PESTICIDE AVOIDANCE BEHAVIOR IN *ANOPHELES ALBIMANUS*, A MALARIA VECTOR IN CENTRAL AND SOUTH AMERICA

1995

CHAREONVIRIYAPHAP

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

GRADUATE EDUCATION
(301) 295-3913

APPROVAL SHEET

Title of Dissertation: "Pesticide Avoidance Behavior in *Anopheles albimanus*, a malaria vector in Central and South America"

Name of Candidate: Theeraphap Chareonviriyaphap
Doctor of Philosophy Degree
11 July 1995

Dissertation and Abstract Approved:

L. Lance Sholdt	July 13, 1995
Committee Chairperson	Date
Donald R. Roberts	July 13, 1995
Committee Member	Date
Claudia J. Golenda	July 13, 1995
Committee Member	Date
Richard G. Andre	July 13, 1995
Committee Member	Date
John [illegible]	July 13, 1995
Committee Member	Date
Susan G. Langreth	July 13, 1995
Committee Member	Date

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

"Pesticide Avoidance Behavior in *Anopheles albimanus*, a malaria vector in Central and South America"

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.

T. Chareonviriyaphap

Theeraphap Chareonviriyaphap
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences

ABSTRACT

Pesticide avoidance behavior in Anopheles albimanus, a malaria vector in Central and South America

Theeraphap Chareonviriyaphap, Doctor of Philosophy, 1995

Dissertation directed by: Donald R. Roberts, Ph.D., Professor, Department of Preventive Medicine and Biometrics

The biological effects of permethrin, deltamethrin and DDT on two laboratory colonies (Santa Tecla colony from El Salvador and El Semillero colony from Guatemala) and two field populations (Toledo and Corozal, Belize) of Anopheles albimanus were characterized by behavioral responses and susceptibility diagnostic tests. The susceptible laboratory colony (Santa Tecla) has been maintained at least 20 years and is susceptible to all three insecticides. The colony from Guatemala was recently colonized and exhibited resistance to permethrin, deltamethrin, and DDT. The third population ("wild-caught") was from southern Belize and demonstrated resistance to DDT only. The fourth population ("wild-caught") was from northern Belize and was susceptible to all three chemicals. Each behavioral study compared escape responses of each test population from a chamber affording direct contact with insecticide-treated surfaces or from a chamber that excluded direct contact with treated surfaces. Two control chambers (one with and one without direct contact with surfaces) treated with the carrier (Risella Oil) only were included in each study. Test chambers affording direct contact with treated surfaces showed a very dramatic escape response of Guatemalan and Belizean mosquitoes for all three chemicals. Numbers of females escaping from chambers without direct contact with treated surfaces were significantly greater than number escaping from control chambers ($P < 0.05$) but less than

numbers escaping from chambers affording direct contact with insecticide. In marked contrast, few females from the susceptible laboratory colony escaped from test chambers, regardless of insecticide used. Isozyme analysis suggested that the susceptible laboratory colony has lost some genetic heterogeneity compared to field-caught and recently colonized populations. Comparison of esterase activity between pyrethroid susceptible and resistant populations was performed using a microtitre plate assay. Comparatively high activity of esterase was found in resistant populations compared to the susceptible populations. This suggests that the presence of this elevated esterase in the Guatemalan colony may limit the use of pyrethroids in malaria control. We conclude that all test populations were representative of a single species, that there is no genetic linkage between genetic susceptibility to the three insecticides and avoidance behaviors exhibited by test populations, and that studies with colonized populations should not be viewed as reflecting how natural populations will respond to insecticides. Finally, we conclude that behavioral responses of malaria vectors are an important aspect in the insecticide-malaria control equation and that this aspect warrants further study in the laboratory and the field.

Pesticide avoidance behavior in Anopheles albimanus, a malaria vector in Central and South America

by

Theeraphap Chareonviriyaphap

Dissertation submitted to the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy 1995

ACKNOWLEDGMENTS

I would like to express my appreciation to the Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences (USUHS), Bethesda, Maryland, USA for the financial support during this research project. Sincere gratitude is extended to my major advisor, Dr.Donald Roberts, for his valuable advice, expert guidance, encouragement and understanding during the various stages of this entire project. He introduced me to the fascinating world of entomology which will guide my own future career.

I am deeply indebted and grateful to Dr. Richard Andre who introduced me to USUHS in 1990 and provided guidance during my doctoral program. Sincere appreciation is also due to Dr.Sylvie-Manguin Gagarine for her kindness and encouragement during my entire study. Special appreciation also to Drs. John Cross, Kenneth Dixon, Lance Sholdt, Claudia Golenda, and Susan Lengreth for their helpful suggestions and guidance during the writing of this manuscript.

Thanks are also due to LCDR. Michael Bangs and Dr. Alan Lindquist for their suggestions in English usage which enabled me to complete this thesis and Dr. Paul Hsieh from Division of Biostatistic, USUHS for his valuable advice on data analysis.

Special thanks are due to Ms. Shilpa Hakre for her assistance during the entomological field trip in Belize, Central America during 1994 and 1995. I would have been faced with more difficulty without her help. Thanks are also due to Ms. Linda Reyes, and Mr. Ernest Black, staff at the Epidemiological Research Center, Belize for their excellent collaboration during my entomological field work. I am deeply grateful to my parents and all my brothers and sisters for their love and endless support during my study at the Uniformed Services University of the Health Science, Bethesda, Maryland, USA.

To someone.....

Silence and I are friends
like blade of grass.
It tells me nothing
and I understand it all.

William Walter Debolt, January 1990

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 4.

CHAPTER 5.

LIST OF FIGURES

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The roles of behavioral responses of An. albimanus to insecticides have been little studied and poorly understood; whereas, the toxicological responses have been studied using tests such as esterase assays and isozyme electrophoresis. Behavioral responses are not easily studied and require several mosquito populations for comparison purposes. The behavioral responses of An. albimanus to insecticides are unquantified while the problems of physiological insecticide resistance in An. albimanus are well known. The studies reported here attempt to clarify the behavioral responses of An. albimanus to DDT and two synthetic pyrethroids, permethrin and deltamethrin, concentrating on the avoidance behavior (sometimes referred to as "behavioral resistance"). The behavioral escape response of An. albimanus to DDT and synthetic pyrethroids is explained in terms of types of action, irritability (direct contact) and repellency (non-direct contact), using special experimental escape chambers. Background information is presented on the current malaria situation, An. albimanus, insecticides, insecticide resistance, physiological resistance, behavioral responses to insecticides, electrophoresis and microplate test methods.

1. Malaria situation

Malaria is currently one of the most important vector-borne diseases in the tropics (Bruce-Chwatt, 1985). Today, the world's population is estimated at around 5.4 billion people with 3.2 billion (58%) living in areas where malaria has never existed or where malaria has been eradicated. However, another 1.8 billion (33%) live in endemic areas where transmission has resumed. These endemic areas include zones where the most severe malaria problems have resulted from major ecological or sociological changes such as exploitation of jungle areas and socio-political unrest. One-half billion people (9%) live in the areas where endemic malaria remains unchanged and where anti-malaria control

programs have utterly failed or never existed, particularly in tropical Africa (World Health Organization, 1994a, 1994b, 1994c).

In 1993, an estimated 290 million (39.2%) people lived in malarious areas in the Americas. Malaria morbidity decreased from 163.6 cases per 100,000 population in 1992 to 132.9 cases per 100,000 population in 1993. In general, 47.4% of all malaria cases were reported from Brazil, 31.67% were from Bolivia, Colombia, Ecuador, and Venezuela, and 15.1% were from Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama in 1993. This reduction in malaria morbidity showed a reversal in the epidemiological trend observed from 1974 to 1992 when the malaria rates increased annually. This decline in the malaria rate was partly due to the changes in epidemiological surveillance methods adopted by countries of the region after the 1987 Directors-General and Directors of malaria programs meeting in Colombia. Decisions were made to focus malaria prevention and control on the highest risk areas using ecological, social, and economic indicators, making it possible to obtain precise malaria data in every locality and tailor programs to meet their specific needs (Pan American Health Organization, 1994).

Today, the number of malaria cases remains unacceptably high in many areas of Latin America. In certain receptive areas, malaria has resurged as a major health problem in Central America, despite a decline in malaria rates in many countries in 1993 including Mexico, Guatemala, Costa Rica, El Salvador, Honduras, and Panama (Pan American Health Organization, 1994). The apparent reduction in malaria incidence has been attributed to implementing new strategies and strengthening existing control programs. For example in Mexico, responding to a greatly increased house spray program, malaria cases declined from 116,238 to 15,793 from 1988 to 1993. In Guatemala, malaria cases decreased from 57,892 to 41,864 from 1991 to 1993. The decrease of malaria cases in Guatemala correlates with an increase in numbers of houses being sprayed for malaria control. In Belize, despite a significant reduction in malaria attack rates in these two neighboring countries, malaria cases have increased dramatically. There was almost a two-

fold increase in malaria cases between 1992 and 1993 in Belize. The increase in malaria has been attributed to greater surveillance and decreased malaria control activities. Most cases continue to be among rural people who are economically depressed with limited access to public health services.

1.1 Anopheles albimanus

Anopheles albimanus Wiedemann (Diptera: Culicidae) is a neotropical species in the subgenus Nyssorhynchus and is considered one of the principal malaria vectors in Central and northern South America (Dyar, 1922; Rodriguez & Loyola, 1989). Anopheles albimanus is widely distributed throughout the tropics and subtropics of the Americas (Figure 1). Its range extends from the Lower Rio Grand Valley of Texas and Florida through the Caribbean region and Central America to Colombia, Ecuador, Venezuela and the Greater Antilles in the south (Faran, 1980; Darsie & Ward, 1981; Bruce-Chwatt, 1985; Ramsey et al., 1988). Although An. albimanus is considered a single species throughout this extensive area, variations in habitat preferences, host seeking behavior, sporozoite rate, and morphology have been reported (Breeland 1972; Garrett-Jones et al., 1980). Arredondo-Jimenez et al. (1992) proposed possible subgroups within the general population that have a preference for human hosts, either by a genetic imprint of host seeking behavior or by learning. Such subgroups may be responsible for malaria transmission (Arredondo-Jimenez et al., 1992). Warren et al. (1975) reported a high level of genetic complexity in a single strain of An. albimanus within a small geographic area of El Salvador. However, studies on Plasmodium susceptibility and refractory strains (Collins et al., 1976), ribosomal DNA (rDNA) analysis (Beach et al., 1989a), analysis of polytene chromosomes (Narang et al., 1991), isozyme frequencies (Narang et al., 1991), hybridization crosses (Narang et al., 1991), egg morphology (Rodriguez et al., 1992) and genetically determined host preferences (Arredondo-Jimenez et al., 1992) showed no cryptic species within this population.

Anopheles albimanus generally occurs in high densities (Rodriguez et al., 1992). In Mexico and the Central American regions, high densities of An. albimanus do not seem to correlate well with malaria rates in human populations (Bown et al., 1991). In the Greater Antilles, Cuba, Jamaica, Puerto Rico and the island of Hispaniola, An. albimanus is widespread in irrigated areas where its larvae are found in stagnant bodies of water in sugar cane fields and rice fields. The larvae of An. albimanus are generally found in sunlit fresh or brackish waters, puddles and marshes, ranging in size from ponds to lagoons. Sunlit pools containing floating or grassy vegetation are particularly favorable sites (Bruce-Chwatt, 1985; Shono et al., 1991; Savage et al., 1990; Rejmankova et al.,1993).

The flight range of a mosquito vector is an important determinant in its ability to disseminate and transmit diseases. The flight range of An. albimanus in Panama, determined by a mark and release study, showed a maximum range of 1.8 km (Zetek, 1915). However, the dispersal of An. albimanus was reported to be over 19 km in Panama (Curry, 1934). In El Salvador, Hobbs et al. (1974) found that An. albimanus females could be recaptured up to 3 km from the release point during the dry season, but the average distance was only 0.5 km. Lowe et al. (1975) reported that the average dispersal distance of An. albimanus, marked with fluorescent powders, was 0.5 km in El Salvador during the dry season and 1 km in the wet season.

Overall, An. albimanus is considered more zoophagic than anthropophagic, more exophagic than endophagic, and more exophilic than endophilic (Breeland, 1972). The general diurnal resting sites of the adults are shaded and protective crevices or natural shelters. Breeland (1972) found that An. albimanus females rested during daylight hours, moved out of resting places at dusk and were active during early evening hours. In El Salvador, the peak period of An. albimanus activity was from 6 to 9 PM with a decrease in activity throughout the remainder of the night (Rachou et al., 1965). In Colombia, this species has a biting peak slightly before midnight (Elliott, 1968). In Peru, An. albimanus was shown to have two biting peaks, one at 6 to 8 PM and the other at 4 to 6 AM (Elliott,

1972). Breeland (1972) has observed that blood-fed females return to their outdoor resting sites after feeding. These resting populations peaked between midnight and 2 AM. This pattern indicates a heightened period of activity in the early evening immediately after dark. In Haiti, An. albimanus was active in the early evening and showed marked exophilic tendencies after feeding (Hobbs et al., 1984).

Colonies of An. albimanus fed on bovine blood under laboratory conditions produced 145 eggs/female (Bailey et al., 1979). Briegel & Rezzonico (1985) reported that the fecundity of An. albimanus increased from 52 to 185 eggs per female when nulliparous females have fed for three consecutive blood meals during the oviposition cycle. Peak oviposition for human and animal-bait collected populations occurred between three and four days post-blood meal under laboratory conditions (Ramsey et al., 1988). From the same study, results showed that the mean oviposition time was slightly longer when mosquitoes fed on human blood than on rabbit blood.

Length of the gonotrophic cycle is important for estimating whether the vector is physiologically old enough to be an effective vector. According to Ramsey et al. (1988), the mean length of the gonotrophic cycle of An. albimanus was 4.7 days post-blood-meal under laboratory conditions at 27°C. This was consistent with Rodriguez et al. (1992) who found field populations of An. albimanus with a gonotrophic cycle of 4 days. Rodriguez et al. (1992) also found a gonotrophic cycle as short as 48 hours. This indicated that, depending on parity status, An. albimanus had 2 gonotrophic cycles, one of 48 hours for parous females and one of 4 days for nulliparous females. Furthermore, multiple blood meals within one gonotrophic cycle were also reported in nulliparous An. albimanus and up to 5 partial blood meals were taken in 6-24 hours. The first blood meal resulted in the maturation of only a few follicles; however, vitellogenesis and fecundity increased with successive blood meals. This indicates the gonotrophic cycle in An. albimanus may be in discordance to blood feeding patterns producing irregular oviposition cycles (Briegel & Horler, 1993).

Several techniques have been used to obtain maximum hatching of An. albimanus eggs in the laboratory. Early hatching investigations were conducted using eggs placed directly on water (Ford & Green, 1972). However, Bailey et al. (1979) and Dame et al. (1978) demonstrated a capacity for eggs of An. albimanus from El Salvador to withstand short-term drying, and this was later used to mass produce and synchronize pupal production (Bailey et al., 1979; Dame et al., 1978). Ramsey et al. (1988) found that eggs collected from El Salvador were very sensitive to hatching temperature. No eggs hatched within 24 hours at 25°C, but 23% of eggs hatched within 24 hours between 25° and 30°C.

Variation in susceptibility to malaria parasites also was reported among several strains of An. albimanus (Chan et al.,1994). The susceptibility of four strains of An. albimanus from El Salvador and one strain from the Republic of Panama to Plasmodium falciparum (Welch) was variable, with generally low numbers of oocysts produced and few sporozoites entering the salivary glands compared to An. maculatus Theobald from Malaysia and An. dirus Peyton & Harrison from Thailand (Collins et al., 1977). This study suggested that not all oocyst-positive mosquitoes allowed complete migration of sporozoites to the salivary glands. In Colombia, the oocyst density in midguts and the sporozoite rate in salivary glands in the Cartegena strain of An. albimanus was low. Low oocyst and sporozoite rates may be partly due to the quality of the gametocytes ingested by the mosquitoes rather than to low vector potential (Olano et al., 1985). In Guatemala, the natural sporozoite antigen rates in 14,150 adult An. albimanus (detected by enzyme-linked immunosorbant assay) were 0.03-0.5% (Beach et al., 1992). In El Salvador, three morphologically distinguishable phenotypes (based on pupal morphology) were found in this species, and they differed significantly in their malaria susceptibility to P. falciparum and P. vivax (Grassi & Feletti) (Warren et al., 1977).

Recently, three morphologically different pupal phenotypes (green, striped and brown) from southern Mexico were found, and these three phenotypes showed significant

differences in susceptibility to P. vivax (Chan et al., 1994). The striped phenotype females had a greater susceptibility to P. vivax than females of the brown and green phenotypes.

2 Insecticides

2.1 DDT

DDT (Dichloro-Diphenyl-Trichloroethane), known also under the name of dicophane or chlorophenothane, was first synthesized as early as 1854; however, its insecticidal effectiveness was not discovered until 1939 by Paul Mueller (Bruce-Chwatt, 1985). The patent application was first made in Switzerland in 1940 (West & Campbell, 1946). Historically, the primary anti-vector intervention against malaria has been spraying of houses with DDT or other residual insecticides (Davidson & Zahar, 1973). During the Second World War, the first field trials of DDT were conducted (Gahan et al., 1945; Metcalf et al., 1945) by the Orlando Laboratory of the United States Bureau of Entomology and Plant Quarantine. DDT was used as an anopheline larvicide and for control of adult mosquitoes beginning in early 1943 (Gahan et al., 1945). DDT was used in the malaria eradication campaign, but currently, DDT is being replaced by newer synthetic compounds.

DDT is a white, amorphous, waxy powder with an aromatic smell. It is insoluble in water but readily soluble in oils and organic solvents. DDT is very stable on suitable impervious surfaces. DDT has been shown effective as a residual surface spray that either kills mosquitoes after prolonged contact or causes an excito-repellency response. Its toxicity is higher on unpainted surfaces or those painted with cold water-casein paint than on surfaces painted with oil-based paint (Gahan et al., 1945). DDT tends to lose its insecticidal activity more quickly on sorptive surfaces like mud (Bruce-Chwatt, 1985).

Aedes aegypti Linnaeus and An. atroparvus van Thiel, displayed distinct behavioral responses after contact with DDT (Kennedy, 1947). Both species exhibited

excitation, ataxia, convulsions, paralysis and finally death. Excitation occurs quickly, often in a matter of seconds after mosquito contact. However, it was observed that the mosquitoes which were knocked down after prolonged contact with low density deposits of DDT recovered within 24 hours and survived at least 48 hours (Kennedy, 1947).

Mode of action

The precise mode of action of DDT toxicity is still unclear. However, the primary effect of DDT on nerve transmission is known (Oppenoorth, 1984). The deleterious effect on the mosquitoes results from excessive excitation in the axons of particular neurons. The axon carries a message, frequently for long distances, without changes in the action potential as it moves along. The action potential of an axon usually has a value of 60-70 mV, the inside being negatively charged with respect to the outside of the membrane. When the nerve is excited, the membrane is depolarized with sodium ions flowing in, resulting in a rapid change of membrane potential opposite the resting membrane potential. Soon afterwards the increased sodium conductivity decreases and potassium conductivity increases, resulting in the rapid recovery of the resting membrane potential (-70mV). The effect of DDT is to delay sodium inactivation by allowing the sodium gate to stay open longer and, to a lesser extent, to suppress potassium ion permeability in the nerve cell. These dual effects cause delayed recovery of action potential of affected nerves. Since the affected axons do not recover to their full resting state, the decreased threshold causes hyperexcitability leading to tremors and eventually paralysis. This paralysis is believed to be the cause of death in poisoned insects (O'Brien, 1978).

Sensory receptors are also affected by DDT. It was reported that DDT had a specific effect on chemoreceptors (Smyth & Roys, 1955) and on sensory hairs, perhaps causing irritability (Soliman & Cutkomp, 1963). It was suggested that the molecular structure of DDT analogues plays an important role in determining their insecticidal action on sense organs (Hadaway & Barlow, 1953).

2.2 Synthetic pyrethroids

Dried pyrethrum flowers were used to control body lice in the early 1800's by Caucasian tribes, and the flowers were first marketed as an insecticide in Armenia in 1828 (Casida, 1980). Production of insect powder began in Yugoslavia and Japan, and eventually moved to east Africa. The majority of the world's current production comes from Kenya, Tanzania, Rwanda, and Ecuador. Insect powder made from the flowers was first imported into the United States in the early 1860's but was not a popular or well-known product. For the last 70 years, liquid sprays have been formulated that are more effective than powdered forms (Casida, 1980).

In the early 1920's, the structure of the natural pyrethroids was elucidated, opening the way for research on synthetic analogues. Allethrin, the first synthetic analogue used in public health, was synthesized in 1949 and commercialized soon after. These so-called 'first generation' pyrethroids tend to decompose rapidly on direct exposure to sunlight. Consequently, they are practical for use as space sprays or other applications requiring rapid degradation of the active ingredient soon after application. The high insecticidal potency of pyrethroids, combined with a low oral toxicity to mammals and rapid biodegradation have made these compounds desirable for many public health uses. The second and third generation synthetic pyrethroids are also desirable for many public health uses and are more photostable and retain longer residual activity. They are especially useful where a relatively persistent insecticide is required for indoor residual spray to control malaria vectors (Anonymous, 1988). Synthetic pyrethroids have been increasingly used since 1986 in malaria control programs (Bisset et al., 1991) .

2.2.1 Permethrin

Permethrin, [3-phenoxybenzyl (1RS, 3RS: 1RS, 3RS)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate, (approx. 60% trans, 40% cis isomers)] is marketed under a variety of names: Ambush, BW-21-Z, Cellutec, Dragnet, Ectiban, Eksmin, FMC-

33297, Indothrin, Kafil, NRDC 143, Permasect, PP 557, Perthrine, Pounce, Pramex, Outflank and Talcord (Farm Chemicals Handbook, 1993). Permethrin has a low mammalian toxicity compared to another common pyrethroid, deltamethrin. When used as a residual spray, permethrin remains effective for at least 3 months (Bruce-Chwatt, 1985).

2.2.2 Deltamethrin

Deltamethrin, [(s)-d-cyanon-phenoxybenzyl (1R, 3R)-3 (2,2-dibromovinyl)-2, 2-dimethyl-cyclopropane carboxylate)] is marketed under the names: Decis, Butoflin, Butox, K-Othrin, NRDC 161 and RU 22974 (Farm Chemicals Handbook, 1993). This compound has a high mammalian toxicity compared to other synthetic pyrethroids. It is effective as an anti-malarial residual spray and retains activity for at least 2 months (Bruce-Chwatt, 1985).

Mode of action

Synthetic pyrethroids are neurotoxins, producing lesions in the motor nerve terminals of several insect species, and can act on both the peripheral nerves and on the central nervous system (Miller, 1988). Like DDT, they can interact at the molecular level with sodium channels by delaying sodium inactivation and suppressing potassium permeability (O'Brien, 1978). In addition, synthetic pyrethroids can also interact with GABA-mediated chloride channels and membrane-bound ATPases (Miller, 1988).

3. Resistance

Resistance is defined as "the developed ability in a strain of insects to tolerate doses of insecticides which prove lethal to the majority of individuals in a normal population of the same species" (Anonymous, 1957). This ability is brought about by selection of individuals in a population with a genetic and inheritable capacity to withstand insecticides, and not due to the action of the insecticide on the individual insect. Therefore, the

development of resistance is dependent on genetic variability already present in a population or arising during the period of selection (Oppenoorth, 1984). Development of physiological insecticide resistance by mosquitoes was first reported in 1947 when Ae. taeniorhynchus (Wiedemann) and Ae. sollicitans (Walker) were shown to be resistant to DDT in Florida (Brown, 1986) after four years of use. Recently, resistance to insecticides has been recorded in 504 species of arthropods (Georghiou & Lagunes, 1991). Most resistance is documented after failure to control a pest, rather than as an early warning of problems to come (Georghiou, 1991). At least 109 mosquito species are resistant to organochlorines, primarily DDT and dieldrin (Brown, 1986).

There are two types of responses to insecticides, one is physiological (or biochemical) and the other is behavioral avoidance[1] (Roberts & Andre, 1994). Behavioral avoidance is the ability of a mosquito to avoid the insecticide-treated surfaces by either contact irritancy or non-contact repellency. Physiological resistance, sometimes referred to as biochemical resistance, is the ability of mosquitoes to survive the effect of insecticide by physiological mechanisms such as detoxifying enzymes (World Health Organization, 1975).

Resistance mechanisms in arthropods have been reported (Georghiou, 1986) to include; reduced sensitivity of altered acetylcholinesterases to organophosphates and carbamates; the kdr (knock down resistance) insensitivity in DDT and pyrethroids; reduced neuronal sensitivity to chlorinated cyclodienes; increased metabolism by hydrolysis to organophosphates, carbamates, and pyrethroids; mixed function oxidase in DDT, organophosphates, carbamates, and pyrethroids; enhanced metabolism by glutathion-S-transferases in organophosphates; enhanced metabolism by DDT-ase in DDT; and reduced

---

[1] "The term behavioral resistance can be defined as a population-based change in a species' genetics, resulting from selective pressure of insecticide use, that increases the frequency of insecticide avoidance behavior" (Roberts & Andre, 1994)

cuticular penetration in DDT, organophosphates, carbamates, pyrethroids, and chlorinated cyclodienes (Kerkut & Gilbert, 1985). Moreover, behavioral avoidance to insecticides should be included in the list (Nolan, 1985) since kdr and pen are considered as the resistance factors for behavioral resistance in the house fly, Musca domestica Linnaeus (Virgona et al., 1983).

Multiple resistance to organochlorine, organophosphate, carbamate, and pyrethroid insecticides has been reported in Ae. aegypti (Linnaeus), Cx. pipiens Linnaeus, Cx. quinquefasciatus Say, An. albimanus , An. culicifacies Giles, An. pseudopunctipennis Theobald, An. sacharovi Favre and An. stephensi Liston (Brown, 1986). Of 58 species resistant to organophosphate insecticides, 54 species were resistant to organochlorines, 17 species were resistant to carbamates (propoxur or bendiocarb) and 10 species were resistant or were characterized with cross-resistance to certain pyrethroids (Brown, 1986).

Anopheles albimanus from most of the coastal areas of Central America, including the Caribbean region, are resistant to almost all known public health insecticides (Brown & Pal, 1971; Brogdon et al., 1988). This high prevalence of resistance is probably due to the large quantities of organochlorine and organophosphorus insecticides that have been used in this region to control insect pests of cotton. The first record of organochlorine multiresistance (DDT and dieldrin) in An. albimanus in Central America occurred in the late 1950s (Davidson, 1963). Anopheles albimanus populations in Guatemala and Nicaragua were found to be resistant to organophosphate and carbamate insecticides (Davidson & Zahar, 1973; Brogdon et al., 1988). Recently, resistance of An. albimanus to synthetic pyrethroids has been reported in Guatemala (Brown, 1986).

Efforts have been made to prevent or slow the development of insecticide resistance or to manage the impact of resistance to new insecticides (Brattsten et al., 1986). Countermeasures have been proposed for avoiding the development of insecticide resistance in mosquitoes (Georghiou, 1980; Brown, 1981; Leeper et al., 1986; Plapp, 1986; Croft, 1990). Countermeasures include varying the doses of insecticide applied,

using local rather than wide area application, applying insecticides only when endemic vector-borne diseases occur, using less persistent insecticides, rotating insecticides and preserving the natural enemies of vectors to minimize the selection pressure from insecticides (Brown, 1981; Roberts & Andre, 1994).

3.1 Physiological Resistance

Insect populations may survive the effect of insecticides by different physiological mechanisms. These include alterations of the site of action, increased detoxification by enzymes, and storage of insecticide in fat body. The first two categories are of importance. Metabolic mechanisms involve a limited number of enzyme systems. Resistance may occur by increasing the quantity of these enzymes in order to break down the insecticides. Hemingway (1991) suggested that two target sites for the commonly used insecticides are acetylcholinesterase for organophosphates and carbamates, and sodium channels for DDT and pyrethroids. Presently, biochemical techniques are available to detect the quantitative changes in esterase, glutathion-s-transferase and acetylcholinesterase in individual insects (Hemingway, 1991). Starch gel electrophoresis is one method for detecting and identifying elevated esterase (Georghiou, 1986).

3.1.1 Physiological resistance to DDT

The major mechanism of DDT resistance is controlled by the kdr (knock down resistance) gene, first identified in the house fly, Mu. domestica (Sawicki, 1973; Farnham, 1977). This mechanism has a direct or indirect impact on the nerve cells and makes the site of action less vulnerable (Oppenoorth, 1984). It involves an alteration in the neural target site which confers resistance both to pyrethroids and DDT (Farnham, 1977; Sawicki et al., 1986).

Biochemical studies have shown that enzyme detoxification is also involved in DDT resistance. Georghiou & Pasteur (1978) reported that dehydrochlorinase is a major

factor in DDT resistance in Cx. quinquefasciatus. This study indicated that DDT may play an important role in changing both oxidase and glutathion (Amin, 1989).

The first case of physiological resistance to DDT in Anopheles was reported in An. saccharovi from Greece after several years of residual spraying (Garret-Jones & Gramiccia, 1954). After 1954, resistance to DDT was recorded in An. hyrcanus Reid and An. pulcherrimus Theobald (Ermishev et al., 1977). Today, DDT resistance in malaria vectors has been reported in many countries (Lockwood et al., 1984; Brown et al., 1976; Brown & Brogdon, 1987; Curtis & Lines, 1987). In Africa, An. gambiae Giles has been found to be focally resistant to DDT (World Health Organization, 1992). In India, physiological resistance was extensively detected in An. culicifacies (Brown et al., 1976; Ramachandra, 1984; World Health Organization, 1992). In Central America, An. pseudopunctipennis and An. albimanus have been reported resistant to DDT and dieldrin (Brown & Pal, 1971; Roberts & Andre, 1994). Extensive studies on DDT resistance in An. albimanus was reported from Mexico (Comision Nacional para la Erradicacion del Paludismo, 1971). In Colombia, An. darlingi Root has been reported to be physiologically resistant to DDT (Suarez et al., 1990).

3.1.2 Physiological resistance to synthetic pyrethroids

One resistance mechanism for pyrethroids is the kdr. The nonmetabolic kdr gene was first studied in the housefly, Mu. domestica (Sawicki, 1973; Farnham, 1977). Physiological resistance to pyrethroids is a critical concern for their future use against arthropods. Nine species of Anopheles in the field have been reported to be resistant to pyrethroids (Malcolm, 1988). This includes four important vectors of malaria, An. albimanus, An. stephensi, An. culicifacies and An. sacharovi (Georghiou, 1986). A World Health Organization database contains only two species of Anopheles that are resistant to pyrethroids in the field. Resistance to deltamethrin has been detected in An.

albimanus in Mexico and six localities in Guatemala and An. sacharovi has been found resistant to permethrin in Syria (Malcolm, 1988).

Biochemical techniques for identifying specific detoxification factors, especially the detection of elevated esterase in resistant individual anophelines, are now available (Brown & Brogdon, 1987). Physiological resistance to pyrethroids involves an increase in the detoxifying enzymes, including elevated nonspecific esterases (Brogdon, 1989). Beach et al. (1989b) found in three populations of An. albimanus from Guatemala that elevated esterase levels strongly correlated with the percentage that survived exposure to deltamethrin. Hydrolyzing esterases are widely distributed in various tissues of insects (Zerba, 1988) and are responsible, to a large extent, for resistance to organophosphates, carbamates, and pyrethroids. These esterases are grouped as type A or B on the basis of their preferential hydrolysis of alpha or beta naphthyl acetate (Pasteur et al., 1981; World Health Organization, 1991a).

4. Behavioral response

There is no convincing example of behavioral "resistance" to insecticides in mosquitoes. Georghiou (1972), split behavioral resistance into two categories, stimulus-dependent and stimulus-independent. Stimulus-dependent resistance requires sensory stimulation of the insect in order for an avoidance response to proceed. In general, this form of avoidance enables the insect to detect the toxicant before acquiring a lethal dose (Muirhead-Thomson, 1960; Sacca & Guy, 1960). On the other hand, stimulus-independent resistance does not require direct sensory stimulation of the insect in order for avoidance to occur but involves natural components of exophily or zoophily in which an insect avoids exposure to a chemical by preferential occupation of non-toxic habitats (Byford & Spark, 1987). The World Health Organization (1986) lists these as phenotypic and genotypic

behaviors. Stimulus-dependent behavioral resistance includes both irritability[2] and repellency[3] (Lockwood et al., 1984).

To facilitate the study of behavioral response of malaria vectors to insecticides, excito-repellency test boxes have been developed. This box is analogous to a single house where mosquitoes are able to exit. Personnel of the World Health Organization, (1970) first developed the demountable plywood excito-repellency test box for studying the irritant effect of the insecticides to mosquito vectors. Several studies have been conducted on malaria vectors using the modified World Health Organization excito-repellency test boxes (Bondareva et al., 1986; Ree & Loong, 1989; Pell et al., 1989; Quinones & Suarez, 1989). Rachou et al., (1973) used the plywood experimental escape box that measured 50 cm on a side for studying the escape response to DDT of An. albimanus population in El Salvador. Similar boxes were used to study behavioral responses of An. darlingi to DDT in Brazil (Charlwood & Paraluppi,1978). Roberts et al., (1984) constructed a collapsible excito-repellency test box and carried out tests on behavioral avoidance of An. darlingi to DDT in Brazil. Light proof test boxes were used to study escape behaviors of a laboratory colony of An. gambiae to DDT, bendiocarb and lambda-cyhalothrin (Evans, 1993). Unfortunately, no methods of behavioral study have been widely accepted, suggesting the difficulties of obtaining and analyzing excito-repellency data (Roberts et al., 1984). Furthermore, the conventional excito-repellency test box only provides for direct contact with insecticides and does not test for non-contact repellency.

---

[2] Irritabilty is defined by an insect leaving the immediate toxic environment after contact with a treated surface.

[3] Repellency is defined by an insect leaving the immediate toxic environment without contact with a treated surface.

4.1 Behavioral response in DDT

Some agricultural and medically important insects, including several major malaria vectors, exhibit what has been termed 'behavioral resistance' to DDT (Lockwood et al., 1984). However, the term behavioral avoidance is preferred since development of behavioral changes due to insecticidal selective pressures in nature has not been adequately documented (Muirhead-Thomson, 1960). The behavioral responses of various mosquito vectors have been examined using either specially constructed experimental huts or excito-repellency test boxes. The first field studies on the irritant effect of DDT residual deposits on An. quadrimaculatus Theobald were conducted in 1944 and 1945. The An. quadrimaculatus became irritated after brief contact with treated surfaces, and many quickly exited the DDT treated house without taking a blood meal (Gahan & Lindquist, 1945; Gahan et al., 1945). Subsequent studies found that many An. quadrimaculatus had received a lethal dose and perished within 24 hours (Metcalf et al., 1945). Unfortunately, these studies did not include control houses or other controls for various observations. Moreover, high mortality of An. quadrimaculatus may have been caused by a series of further contacts with the insecticide while attempting to leave a treated building with a tiny single outlet (Muirhead-Thomson, 1960).

In studies of An. albimanus in Panama, Trapido (1954) concluded that wild An. albimanus, lacking re-exposure to DDT for a long period of time, showed the same susceptibility to DDT as those from a laboratory colony with no a history of exposure to DDT. Malaria vectors in some countries have never developed resistance to DDT (Roberts & Andre, 1994). Perhaps the non-resistant population naturally avoids contact with the chemicals, precluding any selection for resistance to DDT.

4.2 Behavioral responses to synthetic pyrethroids

Several insecticides, including synthetic pyrethroids, repel insects (Roberts & Andre, 1994). This can be highly beneficial to pest control operations. A number of

synthetic pyrethroids have a repellent effect for many agricultural and medically important insects (Lockwood et al., 1984). Sparks et al. (1989) reported that pyrethroid resistance in the hornfly and the tobacco bud worm showed several resistance mechanisms, including behavioral adaptation. These synthetic pyrethroids are allethrin, deltamethrin, permethrin, cypermethrin, fenvalerate, as well as bioresmethrin (Rani & Osmani, 1984; Sparks et al., 1989), and they are primarily used in public health control programs (Quelennec, 1988). Since the early 1980s, there has been a burgeoning interest in the use of pyrethroid impregnated bed nets for malaria control.

Mosquito nets impregnated with synthetic pyrethroids protect people suffering from malaria in many parts of the world. In The Gambia, bed nets treated with permethrin decreased malaria mortalities among children (Alonso et al., 1991). It was indicated that bed nets impregnated with permethrin resulted in 70% protection against P. falciparum in Papua New Guinea (Anonymous, 1991).

Mosquitoes may be killed rapidly when they land on pyrethroid-impregnated fabrics (Darriet et al., 1984; Lines et al., 1985; Lines et al., 1987; Snow et al., 1987). Mosquitoes may come in contact with treated nets, become physically irritated by the insecticide, and depart the area, decreasing the time spent searching for a bloodmeal (Hossain & Curtis, 1989; Miller, 1990). Also, mosquitoes might be deterred from entering the house with a permethrin-treated mosquito net (Darriet et al., 1984; Rozendaal et al., 1989), due to the volatile chemicals that are present in the formulation (Lindsay et al., 1991) rather than the permethrin itself, which is comparatively non-volatile (Wells et al., 1986). Lindsay et al. (1991) concluded that permethrin impregnated bed nets reduced mosquito blood feeding 91% compared to untreated bet nets.

In summary, different synthetic pyrethroids used to impregnate bet nets have various effects on particular vector species. Use of impregnated bed nets may deter mosquitoes from entering dwellings, may cause mosquitoes to leave the dwelling more quickly, or may kill the mosquitoes upon contact.

4.3 Interaction of physiological and behavioral resistance

A number of insects exhibit both physiological and behavioral resistance to DDT (Lockwood, 1984). In some cases there is either physiological or behavioral resistance to some compounds, but not both. Most studies show that the physiological and behavioral resistance mechanisms are not mutually exclusive.

The interaction of behavioral and physiological resistance can be examined by comparing the relationship between behavior and physiology (Lockwood et al., 1984). Changes in physiological and biochemical processes may result in different behavioral responses (Georghiou, 1972). These biochemical reactions are under genetic control and are normally regulated by enzymes and other proteins (Alcock, 1979). Virgona et al. (1983) reported that kdr and pen served as the resistance factors in repellency resistance in the house fly (Mu. domestica).

5. Electrophoresis

5.1 Electrophoresis to detect esterases related to insecticide resistance

Multiple forms of esterases exist in a variety of insect tissues, including mosquitoes (Booth et al., 1973; Zerba, 1988). Esterases in mosquitoes are quite important since they are involved in insecticide resistance. Electrophoresis can be used to detect elevated esterases associated with organophosphate resistance in mosquitoes and to identify esterases that may be involved in pyrethroid resistance. Differentiation is based upon the electrophoretic mobility of esterases and their activity towards naphthyl substrate. Elevated esterase levels are the principal mechanism of resistance to organophosphates and carbamates in mosquitoes. An increase of esterase was found to correlate with survival rates in pyrethroid-resistant mosquitoes after exposure to certain chemicals (Beach et al., 1989b).

Several types of esterases (esterase A1, A2, B1, B2, and C2) were found to be responsible for organophosphate resistance in culicine mosquitoes using isozyme electrophoresis (Georghiou, 1986; Bonning et al., 1991; Yebakima et al., 1995). Highly active esterase B2 was present in organophosphate-resistant culicine mosquitoes (Georghiou & Pasteur, 1978). A high activity of esterase B1 was reported in organophosphate resistance of Cx. quinquefasciatus from California (Georghiou, 1986), while an elevated activity of esterase A1 was shown in Cx. pipiens in 1985 from Italy (Bonning, 1991). Esterase A2 was found in organophosphate resistance in Cx. quinquefasciatus from California (Raymond et al., 1987). Recently, a new esterase (C2) was found with high activity in organophosphate resistant Cx. pipiens from the Islands of Martinique (Yebakima et al., 1995). Most esterase studies with anopheline mosquitoes were conducted using a microplate esterase assay, since it is more practical for field use compared to gel electrophoresis (Georghiou, 1986).

5.2 Electrophoresis for the study of population

Isozyme electrophoresis has been used since 1960 for the study of the genetics and evolutionary biology of many organisms. It also has been used to distinguish species and populations of organisms that are difficult to identify by standard morphological methods (Harris & Hopkinson, 1976). This technique has demonstrated value for the study of phylogenetic relationships, for investigating species homologies, and for studying ecological-genetic relationships.

The starch gel electrophoresis technique allows one to study dozens of genes in one specimen simultaneously. It is possible to analyze as many as 30 specimens at the same time on a single starch gel. Many studies on population genetics of insects have been conducted using starch gel electrophoresis (Hilburn et al., 1984; Narang et al., 1991; Manguin et al., 1993; Manguin et al., 1995).

6. Microtitre plate test

Although the World Health Organization method for insecticide susceptibility tests (World Health Organization, 1970; 1975) has been used for more than 30 years, supplementary methods are now available for the detection of biochemical resistance. The microtitre plate technique can detect resistance conferring enzymatic mechanisms rapidly and easily in field populations (World Health Organization, 1991b). There are several different microtitre plate assays which are based on enzyme-substrate reactions that produce visual color differences: therefore, they can be used under field conditions without sophisticated detection equipment (Brown & Brogdon, 1987). Several homogenates at various dilutions can be evaluated simultaneously, and the same insect can be tested for other enzymes (Brogdon, 1989). Serial dilutions of homogenates can be run for each mosquito to measure the relative amount of esterase and the level of resistance.

A non-specific esterase has been demonstrated to hydrolyze naphthyl acetate (NA) (Pasteur & Georghiou, 1981). Elevated esterase was found in a number of mosquitoes resistant to carbamate and organophosphate insecticides, including Cx. tritaeniorhynchus Giles from Japan, Cx. tarsalis from California, Cx. pipiens from France, Italy, Egypt and Kenya, and Cx. quinquefasciatus from Thailand (Georghiou, 1986; Raymond et al., 1991).

A few anophelines that are resistant to organophosphate and carbamate insecticides have shown elevated esterase. High esterase activity was found to be associated with organophosphate resistance in An. subpictus Grassi in Sri Lanka. Elevated esterase in Haitian An. albimanus also was associated with organophosphate and carbamate insecticide resistance (Brogdon et al., 1988). Elevated esterases were reportedly responsible for organophosphate and pyrethroid resistance in An. albimanus from Guatemala (Beach et al., 1989b).

7. Conclusion

Globally, vector control programs are declining and the malaria problem is growing. To reverse these trends, we need a better understanding of how insecticide residues serve to prevent malaria transmission and a better assessment of the practical significance of different mechanisms. Research described in this dissertation will hopefully contribute to these general goals.

REFERENCES CITED

Alcock, J. 1979. Animal Behavior, an Evolutionary Approach. Sinauer Assoc. Inc. Sunderland, MA.

Alonso, P.L., Lindsay, S.W., Armstrong, J.R.M., Conteh, M., Hill, A.G., David, P.H., Fegan, G., de Francisco, A., Hall, A.J., Shenton, F.C., Cham, K. & Greenwood, B.M. 1991. The effect of insecticide-treated bed nets on mortality of Gambian children. Lancet 337: 1499-1502.

Amin, A.M. & Hemingway, J. 1989. Preliminary investigation of the mechanisms of DDT and pyrethroid resistance in Culex quinquefasciatus (Diptera: Culicidae) from Saudi Arabia. Bull. Entomol. Res. 79: 361-366.

Anonymous, 1957. World Health Expert Committee on Insecticides. Seventh Report, WHO Tech. Rept. Ser., No. 125.

Anonymous, 1988. Pyrethroid insecticides in public health. Parasitol. Today. 4: S1-S3.

Anonymous, 1991. Drawing the curtain on malaria. Lancet 337: 1515-1516.

Arredondo-Jimenez, J.I., Bown, D.N., Rodriguez, M.H., Villarreal, C., Loyola, E.G. & Frederickson, C.E. 1992. Tests for the existence of genetic determination or conditioning in host selection by Anopheles albimanus (Diptera: Culicidae). J. Med. Entomol. 29: 894-897.

Bailey, D.L., Thomas, J.A., Munroe, W.L. & Dame, D.A. 1979. Viability of eggs of Anopheles albimanus and Anopheles quadrimaculatus when dried and stored at various temperatures. Mosq. News 39: 113-116.

Beach, R.F., Mills, D. & Collins, F.H. 1989a. Structure of ribosomal DNA in Anopheles albimanus (Diptera: Culicidae). Ann. Entomol. Soc. Am. 82: 641-648.

Beach, R.F., Cordon-Rosales, C. & Brogdon, W.G. 1989b. Detoxifying esterases may limit the use of pyrethroids for malaria vector control in the Americas. Parasitol. Today 5: 326-327.

Beach, R.F., Cordon-Rosales, C., Molina, E. & Wirtz, R.A. 1992. Field evaluation of an enzyme-linked-immunosorbent-assay for estimating the sporozoite rate in Anopheles albimanus. Am. J. Trop. Med. 47: 478-483.

Bisset, J.A., Rodriguez, M.M., Hemingway, J., Diaz, C., Small, G.J. & Ortiz, E. 1991. Malathion and pyrethroid resistance in Culex quinquefasciatus from Cuba: efficacy of pirimiphos-methyl in the presence of at least three resistance mechanisms. Med. Vet. Entomol. 5: 223-228.

Bondareva, N.I., Artem'ev, M.M. & Gracheva, G.V. 1986. Susceptibility and irritability caused by insecticides to malaria mosquitoes in the USSR. Part 1. Anopheles pulcherrimus. Medit. Parazitol. Parazit. Bolezni 6: 52-55.

Bonning, B.C., Hemingway, J., Romi, R. & Majori, G. 1991. Interaction of insecticide resistance genes in field populations of Culex pipiens (Diptera: Culicidae) from Italy in response to changing insecticide selection pressure. Bull. Entomol. Res. 81: 5-10.

Booth, G.M., Connor, J., Metcalf, R.A. & Larsen, J.R. 1973. A comparative study of the effects of selective inhibitors on esterase isozymes from the mosquito Anopheles punctipennis. Comp. Biochem. Physiol. 44B: 1185-1195.

Bown, D.N., Rodriguez, M.H., Arredondo-Jimenez, J.I., Loyola, E.G. & Rodriguez, M.C. 1991. Age structure and abundance levels in the entomological evaluation of an insecticide used in the control of Anopheles albimanus in southern Mexico. J. Am. Mosq. Control Assoc. 7: 180-187.

Brattsten, L.B., Holyoke C.W. Jr., Leeper, J.R. & Raffa, K.F. 1986. Insecticide resistance: challenge to pest management and basic research. Science 231: 1255-1260.

Breeland, S.G. 1972. Studies on the diurnal resting habits of Anopheles albimanus and An. pseudopuntipennis in El Salvador. Mosq. News 32: 99-106.

Briegel, H. & Horler, E. 1993. Multiple blood meals as a reproductive strategy in Anopheles (Diptera: Culicidae). J. Med. Entomol. 30: 975-985.

Briegel, H. & Rezzonico, L. 1985. Concentration of host blood protein during feeding by anopheline mosquitoes (Diptera: Culicidae). J. Med. Entomol. 22: 612-618.

Brogdon, W.G. 1989. Biochemical resistance detection: an alternative to bioassay. Parasitol. Today 5: 56-60.

Brogdon, W.G., Beach , R.F., Stewart, J.M. & Castanaza, L. 1988. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan Anopheles albimanus. Bull. WHO 66: 339-346.

Brown, A.W.A. & Pal, R. 1971. Insecticide Resistance in Arthropods. Second Ed. WHO. Monogr. Ser. No. 38 WHO, Geneva, 491 pp.

Brown, A.W.A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Control Assoc. 2: 123-140.

Brown, A.W.A., Haworth, J. & Zahar, A.R. 1976. Malaria eradication and control from a global standpoint. J. Med. Entomol. 13: 1-25.

Brown, T.M. & Brogdon, W.G. 1987. Improved detection of insecticide resistance through conventional and molecular techniques. Ann. Rev. Entomol. 32: 145-162.

Brown, T.M. 1981. Countermeasures for insecticide resistance. Entomol. Soc. Am. Bull. 27: 198-202.

Bruce-Chwatt, L.J. 1985. Essential Malariology. Second edition. Alden Press Oxford. 452 pp.

Byford, R.L. & Sparks, T.C. 1987. Chemical approaches to the management of resistance horn fly, Haematobia irritans, populations. [In Combating Resistance to Xenobiotics (eds. Ford, M.G., Holloman, D.W., Khambay, B.P.S. & Sawicki, R.M.)] Ellis Horwood, Chichester, UK. 178-180.

Casida, J.E. 1980. Pyrethrum flowers and pyrethroid insecticides. Environ. Hlth. Perspect. 34: 189-202.

Chan, A.S.T., Rodriguez, M.H., Torres, J.A., Rodriguez, M.C. & Villarreal, C. 1994. Susceptibility of three laboratory strains of Anopheles albimanus (Diptera: Culicidae) to

coindigenous Plasmodium vivax in Southern Mexico. J. Med. Entomol. 31: 399-403.

Charlwood, J.D. & Paraluppi, N.D. 1978. The use of excito-repellency boxes with Anopheles darlingi , A. nuneztovari and Culex pipiens quinquefasciatus obtained from area near Manaus, Amazonas. Acta Amazonica. 8: 605-611.

Collins, W.E., Skinner, J.C., Warren, Mc. W. & Richardson, B. 1976. Studies on human malaria in Aotus monkeys. VII. Comparative infectivity of two strains of Plasmodium vivax to Anopheles freeborni, An. maculatus, and 4 strains of An. albimanus. J. Parasitol. 62: 190-194.

Collins, W.E., Warren, Mc.W., Skinner, J.C., Richardson, B.B. & Kearse, T.S. 1977. Infectivity of the Santa Lucia (El Salvador) strain of Plasmodium falciparum to different anophelines. J. Parasitol. 63: 57-61.

Comision nacional para la erradicacion del paludismo, 1971. Localidades en las que se han effectuado estudios de susceptibilidad de los anofelinos al DDT durante el periodo 1967-1971. Estados Unidos Mexicanos.

Croft, B.A. 1990. Developing a philosophy and program of pesticide resistance management. In Pesticide Resistance in Arthropods by R.T. Roush and Tabashnik, B.E. Editors. Chapman and Hall, New York and London.

Curry, D.P. 1934. Breeding of anopheline mosquitoes among aquatic vegetation of Gatun Lake, accompanied by periodic long flights of Anopheles albimanus. So. Med. J. 27: 644-649.

Curtis, C.F. & Lines, J.D. 1987. Insecticides in the management of insect vectors of tropical disease. Insect Sci. Appl. 8: 709-714.

Dame, D.A., Haile, D.G., Lofgren, C.S., Bailey, D.L. & Munroe, W.L. 1978. Improved rearing techniques for larval Anopheles albimanus. Use of dried mosquito eggs and electric heating tapes. Mosq. News 38: 68-74.

Darriet, F., Robert, V., Vien, N.T. & Carnevale, P. 1984. Evaluation of the efficacy of permethrin impregnated intact and perforated mosquito nets against vectors of malaria. WHO mimeographed document (WHO/VBC/84.899), Geneva, 12 pp.

Darsie, R.F. & Ward, R.A. 1981. Identification and geographical distribution of the mosquitoes of north America. Mosq. Syst. Suppl. 1: 1-313.

Davidson, G. & Zahar, A.R. 1973. The practical implications of resistance of malaria vectors to insecticides. Bull. WHO 49: 475-483.

Davidson, G. 1963. DDT resistance and dieldrin resistance in Anopheles quadrimaculatus. Bull. WHO 29: 177-184.

Dyar, H.G. 1922. The mosquitoes of the United States. Proc. Nat. Musium 62: 101-108.

Elliott, R. 1968. Studies on man-vector contact in some malarious areas in Colombia. Bull. WHO 38: 239-253.

Elliott, R. 1972. The influence of vector behavior on malaria transmission. Am. J. Trop. Med. Hyg. 21: 755-763.

Ermishev, Y.V., Kul'Kova T.A. & Polevoi, N.I. 1977. Part II: Study of the sensitivity and irritability of Anopheles mosquitoes to insecticides in northeastern Afghanistan. Med. Parazitol Parazit. Bolezni. 46: 444-447.

Evans, R.G. 1993. Laboratory evaluation of the irritancy of bendiocarb, lambda-cyhalothrin, and DDT to Anopheles gambiae. J. Am. Mosq. Control Assoc. 9: 285-293.

Faran, M. 1980. A revision of the Albimanus section of the subgenus Nyssorhynchus of Anopheles. Contrib. Am. Entomol. Inst. 15: 1-215.

Farm Chemicals Hand Book. 1993. Buyers'guide to application equipment fertilizer trade names, fertilizer dictionary and pesticide dictionary. Stepan Agriculture. Stepan Co.

Farnham, A.W. 1977. Genetics of resistance of houseflies (Musca domestica) to pyrethroids. I. Knockdown resistance. Pestic. Sci. 8: 631-636.

Ford, H.R. & Green, E. 1972. Laboratory rearing of Anopheles albimanus. Mosq. News 32: 509-513.

Gahan J.B., Travis, B.V. & Lindquist, A.W. 1945. DDT as a residual-type spray to control disease-carrying mosquitoes: Laboratory tests. J. Econ. Entomol. 88: 236-240.

Gahan, J.B. & Lindquist, A.W. 1945. DDT residual sprays applied in buildings to control An. albimanus. J. Econ. Entomol. 38: 223-230.

Garrett-Jones, C. & Gramiccia, G. 1954. Evidence of the development of resistance to DDT by Anopheles sacharovi in the Levant. Bull. WHO 11: 865-883.

Garrett-Jones, C., Boreham, P. & Pant, C. 1980. Feeding habits of anophelines (Diptera: Culicidae) in 1971-1978, with reference to the human blood index: a review. Bull. Entomol. Res. 70: 165-185.

Georghiou, G.P. & Lagunes, A. 1991. The Occurrence of Resistance to Pesticides in Arthropods. FAO, Rome, 312 pp.

Georghiou, G.P. & Pasteur, N. 1978. Electrophoretic esterase patterns in insecticide-resistant and susceptible mosquitoes. J. Econ. Entomol. 71: 201-205.

Georghiou, G.P. 1972. Studies on resistance to carbamate and organophosphorus insecticides in Anopheles albimanus. Am. Trop. Med. Hyg. 21: 797-806.

Georghiou, G.P. 1980. Implications of the development of resistance to pesticide: basic principles and consideration of countermeasures. In: Proceedings of seminar and workshop, Barbados, 3-7 November 1980. Pest. Manag. Caribbean; 1980: 116-129.

Georghiou, G.P. 1986. Strategies and tactics for (resistance) management. In Pesticide Resistance. National Academy Press.

Georghiou, G.P. 1991. New development in biochemistry and genetics of vector resistance to pesticides and their relevance to solving control problems. CTD/OPR/EC/91. Expert Committee on Insecticide Resistance, Geneva. March 5-13, 1991; 43 pp.

Hadaway, A.B. & Barlow, F. 1953. Studies on aqueous suspensions of insecticides. Part IV. The behaviour of mosquitoes in contact with insecticidal deposits. Bull. Entomol. Res. 44: 255-271.

Harris, H. & Hopkinson, D.A. 1976. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland Publ. Comp., Amsterdam.

Hemingway, J. 1991. Applicability and cost implications of biochemical methods of detecting resistance mechanisms under field conditions in comparison with standard WHO bioassay tests. Expert Committee on insecticide resistance. WHO Geneva.

Hilburn, L.R., Willis, N.L. & Seawright, J.A. 1984. An electrophoretic comparison of laboratory colonies and natural population of Toxorynchites (Diptera: Culicidae). J. Med. Entomol. 21: 336-343.

Hobbs, J.H., Lowe, R.E. & Schreck, C.E. 1974. Studies of flight range and survival of Anopheles albimanus in El Salvador. I. Dispersal and survival during the dry season. Mosq. News 34: 389-393.

Hobbs, J.H., Sexton, J.D., Jean, Y.S. & Jacques, J.R. 1984. Behavior of Anopheles albimanus in Haiti. Abstract, pp. 193 in New Jersey Mosq. Control Assoc. CDC, Atlanta, Georgia.

Hossain, M.I. & Curtis, C.F. 1989. Permethrin-impregnated bednets: behavioral and killing effects on mosquitoes. Med. Vet. Entomol. 3: 367-376.

Kennedy, J.S. 1947. The excitant and repellent effects on mosquitoes of sub-lethal contacts with DDT. Bull. Entomol. Res. 37: 593-607.

Kerkut, G.A. & Gilbert, L.I. 1985. Comparative Insect Physiology Biochemistry and Pharmacology. V. 12, Insect control. Pergamon Press. 849 pp.

Leeper, J.R., Roush, R.T., Reynolds, H.T. 1986. Preventing or managing resistance in arthropods. In: Pesticide Resistance: Strategies and Tactics for Management, Washington, D.C., National Academy Press, 335-348.

Lindsay, S.W., Adiamah, J.H., Miller, J.E. & Armstrong, J.R.M. 1991. Pyrethroid-treated bednet effects on mosquitoes of the Anopheles gambiae complex in The Gambia. Med. Vet. Entomol. 5: 477-483.

Lines, J.D., Curtis, C.F., Myamba, J. & Njau, R 1985. Tests of repellence or insecticide impregnated curtains, bednets and anklets against malaria vectors in Tanzania. WHO/VBC/85.920. 16 pp.

Lines, J.D., Myamba, J. & Curtis, C.F. 1987. Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. Med. Vet. Entomol 1: 37-51.

Lockwood, J.A., Sparks, T.C. & Story, R.N. 1984. Evolution of insect resistance to insecticides: A reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30: 41-51.

Lowe, R.E., Schreck, C.E., Hobbs, J.H., Dame, D.A. & Lofgren, C.S. 1975. Studies on flight range and survival of Anopheles albimanus Wiedemann in El Salvador. II. Comparisons of release method with sterile and normal adults in wet and dry seasons. Mosq. News 15: 160-168.

Malcolm, C.A. 1988. Current status of pyrethroid resistance in anophelines. Parasitol. Today 4: S13-S15.

Manguin, S., Peyton, E.L., James, A.C. & Roberts, D.R. 1993. Apparent changes in the abundance and distribution of Anopheles species on Grenada Island. J. Am. Mosq. Control Assoc. 9: 403-407.

Manguin, S., Roberts, D.R., Peyton, E.L., Fernandez-Salas, I., Barreto, M., Fernandez Loayza, T., Spinola, R.E., Granaou, R.M. & Rodriguez, M.H. 1995. Biochemical systematic and population genetic structure of Anopheles pseudopunctipennis, vector of malaria in Central and South America. Am. J. Trop. Med. Hyg. In press.

Metcalf, R.L., Hess, A.D., Smith, G.E., Jeffery G.M. & Ludwig, G.W. 1945. Observations on the use of DDT for the control of Anopheles quadrimaculatus. Publ. Hlth. Rep. 60: 753-774.

Miller, J.E. 1990. Laboratory and field studies of insecticide impregnated fibers for mosquito control. Ph.D. dissertation, University of London. 336 pp.

Miller, T.A. 1988. Mechanisms of resistance to pyrethroid insecticides. Parasitol. Today 7: S8-S12.

Muirhead-Thomson, R.C. 1960. The significance of irritability behavioristic avoidance and allied phenomena in malaria eradication. Bull. WHO 22: 721-734.

Narang, S.K., Seawright , J.A. & Suarez, M.F. 1991. Genetic structure of natural populations of Anopheles albimanus in Colombia. J. Am. Mosq. Control Assoc. 7: 437-445.

Nolan, J. 1985. Mechanisms of resistance to chemicals in arthropod parasites of veterinary importance. Vet. Parasitol. 18: 155-166.

O'Brien, R.D. 1978. The biochemistry of toxic action of insecticides. In "Biochemistry of Insects" (M. Rockstein, ed.), Academic Press, New York, 515-539.

Olano, V.A., Carrillo, M.P., De La Vega, P. & Espinal, C.A. 1985. Vector competence of Cartagena strain of Anopheles albimanus for Plasmodium falciparum and P. vivax. Trans. Roy. Soc. Trop. Med. Hyg. 79: 685-686.

Oppenoorth, F.J. 1984. Biochemistry of insecticide resistance. Pestic. Biochem. Physiol. 22: 187-193.

Pan American Health Organization, 1994. Status of malaria programs in the Americas. XLII Report. PAHO, Washington, DC.

Pasteur, N. & Georghiou, G.P. 1981. Filter paper test for rapid determination of phenotypes with high esterase activity in organophosphate resistant mosquitoes. Mosq. News 41: 181-183.

Pasteur, N., Iseki, A. & Georghiou, G.P. 1981. Genetic and biochemical studies of the highly active esterase A and B associated with organophosphate resistance in mosquitoes of Culex pipiens complex. Biochem. Genetics 19: 909-919.

Pell, J.K., Spinney, M.A. & Ward, K.L. 1989. Observations on the behavior of adult Anopheles gambiae encountering residual deposits of lambda-cyhalothrin compared with the other major classes of commercially available insecticides, Proc. Fourth Annu. Conf. Soc. Vector Ecol. Eur. Region, 18 pp.

Plapp, F.W. Jr. 1986. Genetics and biochemistry of insecticide resistance in arthropods: prospects for the future. In: Pesticide Resistance: Strategies and Tactics for Management. Washington, DC., National Academy Press, 74-76.

Quelennec, G. 1988. Pyrethroids in the WHO pesticide evaluation scheme (WHOPES). Parasitol. Today 4: S15-S17.

Quinones, M.L. & Suarez, M.F. 1989. Irritability to DDT of natural populations of primary malaria vectors in Colombia. J. Am. Mosq. Control Assoc. 5: 56-59.

Rachou, R.G., Lyons, G., Moura-Lima, M. & Kerr, J.A. 1965. Synoptic epidemiological studies of malaria in El Salvador. Am. J. Trop. Med. Hyg. 14: 1-62.

Rachou, R.G., Schinazi, L.A., & Lima, M.M. 1973. An intensive epidimiological study of the causes for failure of residual DDT spraying to interrupt the transmission of malaria in Atalaya and Falla, two villages on the coastal plain of El Salvador, Central America. Rev. Bras. Malariol. Doencas Trop. 25: 5-293.

Ramachandra Rao, T. 1984. The anophelines of india. New Delhi: Indian Council of Medical Research. Revised edition.

Ramsey, J.M., Salinas, E., Lopez, J.R., Angel-Cabanas, G.D., Martinez, L. & Bown, D.N. 1988. Laboratory oviposition, fecundity, and egg hatching ability of colonized Anopheles albimanus from Southwestern Mexico. J. Am. Mosq. Control Assoc. 4: 509-515.

Rani, P.U. & Osmani, Z. 1984. Studies on the toxic, repellent and attractant properties of certain insecticides towards the housefly, Musca domestica nebulo. Intern'l Pest Control, 72-75.

Raymond, M., Callaghan, A., Fort, P. & Pasteur, N. 1991. Worldwide migration of amplified insecticide resistance genes in mosquitoes. Nature 350: 151-153.

Raymond, M., Pasteur, N., Georghiou, G.P., Mellon, R., Wirth, M.C. & Hawley, M. 1987. Detoxification esterases new to California in organophosphate resistant Culex quinquefasciatus (Diptera: Culicidae). J. Med. Entomol. 24: 24-27.

Ree, H.I. & Loong. K.P. 1989. Irritability of Anopheles farauti, An. maculatus and Culex quinquefasciatus to permethrin. Jpn. J. Sanit. Zool. 40: 47-51.

Rejmankova, E., Roberts, D.R., Harbach, R.E., Pecor, J., Peyton, E.L., Manguin, S., Krieg, R., Polanco, J. & Legters, L. 1993. Environmental and regional determination of Anopheles (Diptera: Culicidae) larval distribution in Belize, Central America. Environ. Entomol. 22: 978-992.

Roberts, D.R. & Andre, R.G. 1994. Insecticide resistance issues in vectors. Am. J. Trop. Med. Hyg. 50 (suppl): 21-34.

Roberts, D.R., Alecrim, W.D., Tavares, A.M. & McNeill, K.M. 1984. Influence of physiological conditions on the behavioral response of Anopheles darlingi to DDT. Mosq. News 44: 357-362.

Rodriguez, M.H. & Loyola, E.G. 1989. Situation epidemiological actual y perspectives de la investigacion Entogica del paludismo en Mexico, pp. 15-40, Memorias del IV Simposio Nacional de Entogia Medica y Veterinaria. Oaxtepec, Mor., Mexico. Sociedad Mexicana de Entogia.

Rodriguez, M.H., Bown, D.N., Arredondo-Jimenez, J.I., Villarreal, C., Loyola, E.G. & Frederickson, C.E. 1992. Gonotrophic cycle and survivorship of Anopheles albimanus (Diptera: Culicidae) in Southern Mexico. J. Med. Entomol. 29: 395-399.

Rozendaal, J.A., Van Hoof, J.P.M., Voorham, J. & Oostburg, B.F.J. 1989. Efficacy of mosquito nets treated with permethrin in Suriname. Med. Vet. Entomol. 3: 353-365.

Sacca, G. & Guy, Y. 1960. Resistance de comportement au DDT chez An. labranchiae au Moroc. Bull. WHO 22: 735-741.

Savage, H.M., Rejmankova, E., Arredondo-Jimenez, J.I., Roberts, D.R. & Rodriguez, M.H. 1990. Limnological and botanical characterization of larval habitats for Anopheles albimanus and Anopheles pseudopunctipennis in coastal areas of Chiapas State, Mexico. J. Am. Mosq. Control Assoc. 6: 612-620.

Sawicki, R.M. 1973. Resynthesis of multiple resistance to organophosphorus insecticides from strains with factors of resistance isolated from the SKA strain of houseflies. Pestic. Sci. 4: 171-180.

Sawicki, R.M., Farnham, A.W., Denholm, I. & Church, V.J. 1986. Potentiation of Super-kdr resistance to deltamethrin and other pyrethroids by an intensifier (Factor 161) on Autosome 2 in the housefly (Musca domestica). Pestic. Sci. 17: 483-488.

Shono, Y., Jean-Francois, V., Jean, Y.S. & Itoh, T. 1991. Field evaluation of ultra-low volume applications with a mixture of d-allethrin and d-phenothrin for control of Anopheles albimanus in Haiti. J. Am. Mosq. Control Assoc. 7: 494-495.

Smyth, T. Jr. & Roys, C.C. 1955. Chemoreceptor in insects and the action of DDT. Biol. Bull. 108: 66-76.

Snow, R.W., Jawara. M. & Greenwood, B.M. 1987. Observations on Anopheles gambiae Giles s.1. during a trial of permethrin treated bednets in The Gambia. Bull. Entomol. Res. 77: 279-286.

Soliman, S.A. & Cutkomp, L.K. 1963. A comparison of chemoreceptor and whole-fly responses to DDT and parathion. J. Econ. Entomol. 56: 492-494.

Sparks, T.C., Lockwood, J.A., Byford, R.L., Graves. J.B. & Leonard, B.R. 1989. The role of behavior in insecticide resistance. Pestic. Sci. 26: 383-399.

Suarez, M.F., Quinones, M.L., Palacios, J.D. & Carillo, A. 1990. First record of DDT resistance in Anopheles darlingi. J. Am. Mosq. Control Assoc. 6: 72-74.

Trapido, H. 1954. Recent experiments on possible resistance to DDT by Anopheles albimanus in Panama. Bull. WHO 11: 885-889.

Virgona, C.T., Holan, G. & Shipp, E. 1983. Repellency of insecticides to resistant strains of housefly. Entomol. Exp. Appl. 34: 287-290.

Warren, Mc. W., Mason, J. & Hobbs. J. 1975. Natural infections of Anopheles albimanus in small malaria focus. Am. J. Trop. Med. Hyg. 24: 545-546.

Warren, Mc.W., Collins, W.E., Richardson, B.B. & Skinner, J.C. 1977. Morphologic variants of Anopheles albimanus and susceptibility to Plasmodium vivax and P. falciparum. Am. J. Trop. Med. Hyg. 26: 607-611.

Wells, D., Grayson, B.T. & Langner, E. 1986. Vapour pressure of permethrin. Pestic. Sci. 17: 473-476.

West, T.F. & Campbell, G.A. 1946. DDT, the Synthetic Insecticide. London, Chapman & Hall Ltd. 301 pp.

World Health Organization 1970. The WHO standard susceptibility test. WHO Tech. Rept. Ser., No. 443, Annexes 1A, 1B, 2A & 2B.

World Health Organization 1975. Manual on practical entomology in malaria. Part II. method and techniques. Geneva, WHO Offset Publication, No 13.

World Health Organization 1986. Resistance of vectors and reservoirs of disease to pesticides: Tenth Report of the WHO Expert Committee on vector biology and control. No. 737.

World Health Organization 1991a. The mechanisms and significance of gene amplification in mosquito resistance by Raymond and Pasteur. Geneva, 5-12 March 1991. Draft agenda item 6.1. CTD/OPR/EC/91.42, Geneva, Switzerland. 4 pp.

World Health Organization 1991b. New developments in biochemistry and genetics of vector resistance to pesticides and their relevance to solving control problems. Geneva, 5 to 13 March, 1991. CTD/OPR/EC/91.43, Geneva, Switzerland. 8 pp.

World Health Organization 1992. Vector resistance to pesticides. Fifteen Report of the WHO Expert Committee on vector biology and control. WHO Tech. Rept. Ser. 818, 62 pp.

World Health Organization 1994a. Weekly Epidemiological Record 42. World Health Organization Geneva, Switzerland, 309-314.

World Health Organization 1994b. Weekly Epidemiological Record 43. World Health Organization Geneva, Switzerland, 317-323.

World Health Organization 1994c. Weekly Epidemiological Record 44. World Health Organization Geneva, Switzerland, 325-330.

Yebakima, A., Raymond, M., Marquine, M. & Pasteur, N. 1995. Resistance to organophosphorous insecticides in Culex pipiens quinquefasciatus (Diptera: Culicidae) from Martinique. J. Med. Entomol. 32: 77-82.

Zerba, E. 1988. Insecticidal activity of pyrethroids on insects of medical importance. Parasitol. Today 4: S3-S7.

Zetek, J. 1915. Behavior of Anopheles albimanus, and tarsimaculatus. Ann. Entomol. Soc. Am. 8: 221-271.

Figure 1. Geographical distribution of Anopheles albimanus (Darsie & Ward, 1981)

Figure 2. Distribution and insecticide resistance of Anopheles albimanus in Central America and Caribbean Region (Brown & Pal, 1971).

CHAPTER 2

THE TOXICITIES OF DDT AND PYRETHROIDS IN FIVE STRAINS OF *ANOPHELES ALBIMANUS* WIEDEMANN AND THE IDENTIFICATION OF ELEVATED ESTERASE ACTIVITY IN THE PYRETHROID RESISTANT STRAIN

ABSTRACT

The toxicities of permethrin and deltamethrin were determined for a colonized Anopheles albimanus Wiedemann population from El Salvador (Santa Tecla colony) and a recently colonized population from Guatemala (El Semillero colony). Additionally, the toxicity of DDT was tested for the Santa Tecla colony. In adults, the LD_{50} values for permethrin (0.0189 g/m^2) and deltamethrin (0.0007 g/m^2) in the El Semillero colony were 2 times greater than were the LD_{50} values for permethrin (0.0092 g/m^2) and deltamethrin (0.0003 g/m^2) in the Santa Tecla colony. Similarly, the LC_{50} value for permethrin (0.0605 ppm) and deltamethrin (0.0013 ppm) in larvae from the El Semillero colony were two times greater than were the LC_{50} values for permethrin (0.0316 ppm) and deltamethrin (0.0006 ppm) in the Santa Tecla colony. Although there was a two-fold difference in the toxicities of permethrin and deltamethrin between populations, the differences were not statistically significant ($P > 0.05$).

The standardized diagnostic, World Health Organization (WHO) susceptibility test was used to evaluate DDT, permethrin, and deltamethrin on two field populations from northern (Corozal) and southern (Toledo) Belize in addition to the Santa Tecla and El Semillero colonies. The Santa Tecla colony and Corozal population were susceptible to all three compounds while the El Semillero colony showed resistance to all three compounds. The Toledo population was resistant only to DDT.

Total body protein was measured in the Santa Tecla and El Semillero colonies. There was no significant difference between the total protein content of the two colonies, indicating that the degree of pyrethroid susceptibility was not related to mosquito size.

The specific activity of esterase was measured in five populations of An. albimanus. There was a four-seven-fold increase in the specific activity of esterase as measured by the hydrolysis of alpha and beta naphthyl-propionate in the El Semillero colony compared to all the other test populations (Santa Tecla, Belize {Cayo}, Belize

{Toledo}, and Belize {Corozal}). This suggests that the elevated LD_{50} values for pyrethroids in the El Semillero colony may be related to increased esterase activity and the ability of this colony to metabolize pyrethroids more effectively than the other test populations.

Based on these results, permethrin and deltamethrin should be useful for An. albimanus control in Belize. However, the use of DDT in Toledo District warrants close monitoring to ascertain its continued effectiveness.

INTRODUCTION

Resistance to insecticides has been recorded in 504 species of arthropods with most cases of resistance documented only after failure to control a pest (Georghiou, 1991). This includes Anopheles albimanus, one of the most important malaria vectors in Central and South America (World Health Organization, 1992). This species has demonstrated resistance to all major types of insecticides including organochlorine compounds such as DDT; organophosphorus compounds such as malathion and fenitrothion; carbamates such as propoxur and bendiocarb; and synthetic pyrethroids such as permethrin and deltamethrin (Beach et al. 1989). The conventional method for measuring resistance is based on the World Health Organization (WHO) susceptibility test (1975) which requires a comparatively high number of mosquitoes for testing. While this test is indicative of absence or presence of resistance, it provides no information on the underlying mechanisms of resistance.

Susceptibility tests can be complimented by biochemical assays that may give additional information on the underlying mechanisms of insecticide resistance (World Health Organization, 1991). Two biochemical techniques, microplate assay and filter paper test, are often used to evaluate enzyme levels in field populations (World Health Organization, 1991). These tests are based on reactions that produce visual color differences. They can be used under field conditions without sophisticated equipment (Brown & Brogdon, 1987) or can be read in a laboratory with a spectrophotometer. Several insects can be evaluated simultaneously, and the same insect can be tested for other enzymes (Brogdon, 1989). Esterase activity is often evaluated in organophosphate, carbamate, and certain pyrethroid resistant mosquitoes (Cordon-Rosales et al. 1990). For example, elevated esterase was detected in organophosphate and pyrethroid resistant An. albimanus adults using the microtitre plate technique (Beach et al., 1989).

In this study, we determined the toxicity of permethrin and deltamethrin to colonized populations of An. albimanus from El Salvador and Guatemala, and of DDT to the colonized population from El Salvador. Using the WHO diagnostic test, the colonized populations from El Salvador and Guatemala, and two Belizean field-caught populations from Toledo and Corozal Districts were tested for susceptibilities to DDT, permethrin, and deltamethrin. Complementary data were obtained by using the microtiter plate assay to measure the level of whole body esterases in the two laboratory colonies of mosquitoes from El Salvador and Guatemala and three wild-caught populations from Belize.

MATERIALS AND METHODS

Origin of An. albimanus populations

1. Santa Tecla colony (ST colony). This colony was collected from a stable in La Libertad in 1975 and was originally colonized in Santa Tecla, El Salvador. Subsequently, it was colonized at US Department of Agriculture (USDA) in Gainesville, Florida and then at the Walter Reed Army Institute of Research (WRAIR) (Seawright, per comm). We received this colony from WRAIR in 1993 and have maintained it at the Uniformed Services University of the Health Sciences, Bethesda, Maryland.

2. El Semillero colony (ES colony). This colony was originally collected from a cattle corral in El Semillero, the Pacific Coast of Guatemala and the colony has been maintained in the Laboratory of Medical Entomology Research and Training Unit (MERTU/G), Guatemala since 1992. We obtained this colony from MERTU/G in 1993 and maintained it in the insectary at the Uniformed Services University of the Health Sciences. This colony exhibited resistance to permethrin (Cordon-Rosales, per. comm.)

3. Belize-wild caught population from Cayo (CA population). Specimens were collected as larvae in Cayo District, Belize in May 1994.

4. Belize-wild caught population from Toledo (TO population). Specimens were obtained from human landing collections near rice fields in San Filipe Village, Toledo District, Belize in September, 1994.

5. Belize wild-caught population from Corozal (CO population). Specimens were obtained from human landing collections near a marsh area in Chan Chen Village, Corozal District, Belize in February, 1995.

Technical grade insecticides used

1. Permethrin [3-phenoxybenzyl (1RS, 3RS: 1RS, 3RS)-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate] (approx. 60% trans, 40% cis isomers) (94.7%

purity) was kindly provided by Roussel Uclaf Environmental Health (UK) Ltd., United Kingdom in January, 1994.

2. Deltamethrin [(s)-d-cyanon-phenoxybenzyl (1R, 3R)-3 (2, 2-dibromovinyl)-2, 2-dimethyl-cyclopropane carboxylate] (99.7% purity) was also provided by Roussel Uclaf Environmental Health (UK) Ltd., United Kingdom in January, 1994.

3. DDT (Dichloro-diphenyl-trichloroethane) (99% purity). DDT was obtained from the Entomological Sciences Division, United State Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland in October, 1994.

Mosquito rearing

Anopheles albimanus colonies were reared following the methods by Ford & Green (1972), with the following modifications: All life stages were reared in an environmentally controlled ($25 \pm 5^{o}C$, $80 \pm 10\%$RH) insectary at the Uniformed Services University of the Health Sciences, Bethesda, MD. Adult mosquitoes were provided cotton soaked with a 10% sugar solution from the day of emergence. This was changed twice weekly. Female mosquitoes were given a human blood meal on the fourth day post emergence by placing a volunteer's arm into the cage for approximately 20 minutes. Two days later, oviposition dishes were placed in the cage with gravid females for egg laying. Eggs were removed each morning, and immediately transferred into a hatching tray which contained distilled water (2.5 cm depth). The eggs were placed in the center of a piece of wax paper with a hole cut in the center. The paper floated on the water surface and served to keep eggs from becoming stranded on the sides of the hatching tray. First stage larvae were transferred to metal enamel rearing trays containing distilled water (4 cm depth). The water in each tray was allowed to reach room temperature before introducing larvae.

Newly hatched larvae were transferred to rearing trays via a wide-mouthed pipette at densities of 200-300 larvae per tray. Powdered Tetramin^{R} "L" larval food (baby fish food) was sprinkled on the surface of the water twice daily (three times daily after reaching

the third larval stage). Excess food was removed daily to prevent scum by dragging a paper towel across the water surface or changing the rearing water. Pupae were collected daily, and transferred into small bowls containing clean water. Two to four bowls (approximately 100 larvae per bowl) were placed in a screened cage, and the pupae allowed to emerge. Bowls were removed from the cage after adult emergence.

WHO Diagnostic tests

Four populations of An. albimanus adults were exposed for one hour to diagnostic dosages of DDT (4%), permethrin (0.25%), and deltamethrin (0.025%) according to the WHO protocol (World Health Organization, 1981a,b, c and d). For each test, five cylinders (two for controls and three for treatments) were used. Control cylinders contained filter paper impregnated with solvent; whereas, treatments contained paper impregnated with the diagnostic concentration of insecticide in solvent. Twenty mosquitoes were introduced into each cylinder for one hour. Mosquitoes were then transferred to holding containers, and a 10% sucrose solution was provided. Mortalities were recorded at 24 hours. Each test was replicated three times.

Toxicity tests

Anopheles albimanus adults (colonized populations from El Salvador and Guatemala) were exposed to seven concentrations of permethrin (0.0014 to 0.0924 g/m^2) and six concentrations of deltamethrin (0.0002 to 0.0092 g/m^2). Only the colonized population from El Salvador was tested against 4 concentrations of DDT (0.1527, 0.3055, 0.6111, and 1.2222 g/m^2). Insecticide impregnated papers were received from the Entomological Sciences Division, United States Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland. The control cylinder contained paper impregnated with solvent. The treatment cylinders contained paper impregnated with insecticides plus solvent. Twenty mosquitoes were introduced into each

cylinder for one hour. Mosquitoes were then transferred to the holding containers. The holding tubes were kept for 24 hours under temperatures of $25\pm5^{o}C$ and a relative humidity of $80\pm10\%$. Mosquitoes were provided a 10% sucrose solution. Mortalities were recorded at 24 hours. Each test was replicated 4 times.

Larval Anopheles albimanus (colonized populations from El Salvador and Guatemala) were exposed to five concentrations of permethrin (0.001-0.625 ppm) and six concentrations of deltamethrin (0.0007-0.025 ppm) (World Health Organization, 1975). As with adults, only the colonized population from El Salvador was tested against five concentrations of DDT, ranging from 0.003125 to 0.05 ppm.

The insecticides were prepared as stock solutions in 95% ethanol, and serial dilutions were made with distilled water. Each dilution was tested in a 250-ml glass beaker containing a total volume of 100 ml (El-Khatib & Georghiou, 1985). Ten fourth-stage mosquito larvae were placed in each test container. Control (non-exposed) mosquitoes were tested simultaneously. A small applicator stick was used to gently touch moribund larvae to determine whether they were dead or alive. Larval mortalities were recorded at 24 hours. Tests were replicated four times.

Protein analysis

The total protein content of individual An. albimanus specimens was determined using a BioRad protein assay system (Hercules, California). Individual mosquitoes were homogenized in 0.5 ml of phosphate buffer (0.2 mol, pH 7.0) using a plastic microcentifuge tube and pestle. The homogenate was frozen at -70 ^{o}C until the assay was performed. Five microliters were assayed using a microtiter plate technique. Results were compared to a standard curve.

Esterase enzyme assay

The method of Pasteur et al. (1988) and Peiris & Hemingway (1990) were used with the following modification: alpha and beta naphthyl propionate was used in place of alpha and beta naphthyl acetate. The quantity of naphthol produced from the esterase reactions was calculated from standard curves of alpha and beta naphthol. Results were expressed as m-mol product/min/mg protein.

Data analyses

Abbott's formula was used to correct for control mortality within each treatment (lot of test replicates per day). Larvae that pupated during any given test were subtracted from original numbers tested. The LC_{50} and LC_{90} were estimated by probit analysis from percent mortalities obtained with the different concentrations of each chemical (Finney, 1971).

One way analysis of variance (ANOVA) was used to compare the protein content and esterase activity within and among populations. Significance was determined at $P = 0.05$.

RESULTS

The percent mortality of adult An. albimanus at the single diagnostic dosage recommended by WHO (1975) is presented in Table 1. The ability of mosquitoes to survive the diagnostic dose after 24 hours is indicative of resistance in the population; however, as defined by percent mortality in the test population. Based on mortality, the ST colony was susceptible to both DDT and pyrethroids as evidenced by 100% mortality to all three compounds. Similarly, the CO population was susceptible (100% mortality) to all three compounds. The mortality ranged between 45-50% for all three compounds in the ES colony, while the TO population demonstrated some resistance to DDT (65% mortality), but susceptibility to the two pyrethroids.

Based on the LC_{50} values, the toxicities of permethrin and deltamethrin to fourth stage larvae were two times greater for the ST colony than for the ES colony (Table 2 and 3). However, the difference in mortality between the two colonies was not statistically significant for either permethrin [P (LC_{50}) > 0.05 and P (LC_{90}) > 0.05] or deltamethrin [P (LC_{50}) > 0.05 and P (LC_{90}) > 0.05]. Similarly, permethrin and deltamethrin were two times more toxic to adults of the ST colony than to the ES colony, as evidence by LC_{50} values presented in Tables 4 and 5. The differences were not statistically significant between the two colonies for permethrin [P (LD_{50}) > 0.05 and P (LD_{90}) > 0.05] or deltamethrin [P (LD_{50}) > 0.05 and P (LD_{90}) > 0.05].

There is no significant difference, as determined by ANOVA, in the total protein content between ST and ES colonies (P > 0.05) (Table 6). However, a significant difference in the total body protein content was found when laboratory reared mosquitoes (ST and ES colonies) were compared to field mosquitoes (CA, CO, and TO populations) ($P < 0.05$).

Esterase activity in five populations of An. albimanus was measured (Table 6). Elevated esterase was found only in the ES colony. The increase in esterase activity ranged from 4.7 to 7.2 times for alpha-naphthyl propionate and 3.3 to 3.9 times for beta-naphthyl

propionate. ANOVA was used to compare the esterase activity among the five populations for both alpha and beta-naphthyl propionate. The ANOVA indicated that esterase activity in the ES colony was significantly higher than the ST colony and CA, CO, and TO wild-caught populations from Belize ($P < 0.05$). No statistical difference in esterase activity was seen among ST and three field populations (CA, TO, and CO) ($P > 0.05$) (Table 6).

The esterase activity between males and females in ST, ES colonies and CA populations was compared. Only in the ES colony was there a significant difference in the esterase activity between male and female mosquitoes, as determined by an ANOVA ($P < 0.05$). Total protein was significantly different between male and female mosquitoes in the ST and ES colonies ($P < 0.05$), but no significant difference was observed in the CA population ($P > 0.05$) (Table 7).

DISCUSSION

The WHO diagnostic test was performed on four populations of An. albimanus. Based on percent mortality, the ES colony demonstrated resistance to deltamethrin, permethrin, and DDT, while the TO population showed resistance to DDT and susceptibility to the two pyrethroids. The ST colony and CO populations were susceptible to all three compounds.

To determine the level of resistance to the pyrethroids in the ES colony, we conducted dose-response tests on both adult and larval stage mosquitoes, comparing the ES colony to the susceptible ST colony. The latter colony has been maintained in the laboratory for at least 20 years, free of exposure to insecticides. In both the larval and adult stages, the LC_{50} or LD_{50} values for permethrin and deltamethrin in the ES colony were two times higher than were the LC_{50} or LD_{50} values for permethrin and deltamethrin in the susceptible ST colony. Although this difference was not statistically significant, it demonstrated that permethrin and deltamethrin were not as toxic to the ES colony, as to the ST colony. Although dose response tests of DDT were not conducted on the ES colony the results of the WHO diagnostic test suggested resistance to DDT in this colony.

The pyrethroid resistance observed in the An. albimanus colony from Guatemala (ES colony) is consistent with the deltamethrin resistance reported by Malcolm (1988) in six localities and by Beach et al. (1989) in three localities of Guatemala. Pyrethroids have been used since 1986 in malaria control programs in Guatemala (Bisset et al., 1991) and may act as selecting agents. Frequently, pyrethroid resistance occurs in DDT resistant insects as reported for Ae. aegypti from Thailand (Brealey et al., 1984) and Guyana (Prasittisuk & Busvine, 1977). Additionally, pyrethroid resistance in larvae was reported for a DDT selected strain of An. stephensi from Pakistan (Omar et al., 1980). The WHO diagnostic test also indicated that the ES colony was resistant to DDT, suggesting that resistance to synthetic pyrethroids may have arisen from previous use of DDT.

Results of the WHO susceptibility test indicated that the CO population was susceptible to DDT while the TO population was resistant to DDT. DDT was used for malaria control in Toledo, southern Belize, until 1990 when most anti-malarial control was suspended due to a shortage of insecticides. Currently, DDT is being used for malaria control along the Mexican-Belizean border through an inter-country agreement (Vanzie pers. comm.). Indeed, it is interesting that two populations may have had exposure to DDT, yet only one population developed resistance. A clue to reasons for this difference may be related to selection pressure by DDT and DDT-like compounds. In Corozal, Gramoxone, a DDT-like herbicide, was never used for the weed control in rice fields. In Toledo, Gramoxone has been used for weed control. Rice fields are important An. albimanus larval habitats in Toledo. Perhaps continued exposure of both larvae and adults to DDT and Gramoxone resulted in the TO population acquiring resistance.

Pyrethroids are insecticidal esters derived from primary alcohols and are susceptible to hydrolysis by esterases (Kerkut & Gilbert, 1985). Elevated esterase levels have been reported in the pyrethroid resistant southern army worm (Spodoptera eridania) (Abdel Aal & Soderlund, 1980) and the pyrethroid resistant Egyptian cotton leafworm (Spodoptera littoralis) (Riskallah, 1983). Similarly, three deltamethrin resistant populations of An. albimanus from Guatemala have demonstrated elevated esterase levels (Beach et al., 1989). Our study indicated that there was a 4-7 fold increase in hydrolysis of alpha and beta naphthyl propionate to naphthol in whole body homogenates from the ES colony when compared to the ST colony and to the wild-caught CA, CO, and TO populations from Belize that were susceptible to pyrethroids. Perhaps the increase in the LD_{50}s for permethrin and deltamethrin in the ES colony is related to elevated esterase activity in these mosquitoes.

In both ST and ES laboratory colonies, whole body extracts from females contained significantly more protein than extracts from males. This was not true of field-caught CA, CO, and TO mosquitoes from Belize. Additionally, field-caught mosquitoes were

consistently smaller in size than laboratory reared mosquitoes. External factors such as availability and quantity of food during larval development, larval density, and temperature during larval development can affect mosquito size (Clements, 1992). Any or all these factors are more likely to impact on field caught mosquitoes than laboratory colonies.

Ferrari & Georghiou (1990) reported a significantly higher esterase activity in organophosphate resistant Cx. quinquefasciatus males than in females; however, susceptible females demonstrated a significantly higher esterase activity than susceptible males. In our study, ES females had a significantly higher esterase activity compared to ES males. While the reason for this difference is unknown, it could possibly be related to the foraging of females for bloodmeals. In host seeking, females may spend more time in insecticide treated areas such as human and animal quarters. In Guatemala, there has been an increased use of permethrin and deltamethrin for intradomicillary spraying (Beach et al., 1989). The host preference of female An. albimanus is variable and can include domestic animals such as cattle, horses, dogs and swine. Humans are also important hosts for An. albimanus (Breeland, 1972). It has been shown that selection by toxic substances can increase the amount of enzymes that are responsible for detoxification (Ferrari & Georghiou, 1990). Increases in the quantity of enzymes are often associated with gene amplification. Ferrari & Georghiou (1990) reported amplification of an esterase B1 gene in Cx. quinquefasciatus that had high titers of an esterase that confers resistance to organophosphate insecticides. Furthermore, Mouches et al. (1990) reported that gene amplification appeared to be the mechanism causing protein overproduction when an organism is under environmental stress. Female mosquitoes from Guatemala, before colonization, may have been under continual selection pressure when seeking bloodmeals in areas sprayed by pyrethroids or other insecticides. Indeed, this type of selection pressure may have played a significant role in the higher esterase activity observed in the female mosquitoes from Guatemala.

Our study indicated that resistance to the pyrethroids, permethrin and deltamethrin, was represent in a laboratory population that originated from field-collected populations of An. albimanus from Guatemala. This colonized population also exhibits resistance to DDT. The exact mechanism of the pyrethroid resistance in unknown but may be related to elevated esterase activity.

REFERENCES CITED

Abdel-Aal, Y.A.I. & Soderlund, D.M. 1980. Pyrethroid-hydrolysing esterase in southern armyworm larvae: tissue distribution, kinetic properties, and selective inhibition. Pest. Biochem. Physiol. 14: 282-289.

Beach, R.F., Cordon-Rosales, C. & Brogdon, W.G. 1989. Detoxifying esterases may limit the use of pyrethroids for malaria control in the Americas. Parasitol. Today 5: 326-327.

Bisset, J.A., Rodriguez, M.M., Hemingway, J., Diaz, C., Small, G.J. & Ortiz, E. 1991. The mechanisms of organophosphate and carbamate resistance in Culex quinquefasciatus from Cuba: efficacy of pirimiphos-methyl in the presence of at least three resistance mechanisms. Med. Vet. Entomol. 5: 223-228.

Brealey, C.J., Crampton, P.L., Chadwick, P.R. & Rickett, F.E. 1984. Resistance mechanisms to DDT and transpermethrin in Aedes aegypti. Pest. Sci. 15: 121-132.

Breeland, S.G. 1972. Studies on the ecology of Anopheles albimanus. Am. J. Trop. Med. Hyg. 21: 751-754.

Brogdon, W.G. 1989. Biochemical resistance detection: an alternative to bioassay. Parasitol. Today 5: 56-60.

Brown, T.M. & Brogdon, W.G. 1987. Improved detection of insecticide resistance through conventional and molecular techniques. Ann. Rev. Entomol. 32: 145-162.

Cordon-Rosales, C., Beach, R.F. & Brogdon, W.G. 1990. Field evaluation of methods for estimating carbamate resistance in Anopheles albimanus mosquitoes from a microplate assay for insensitive acetylcholinesterase. Bull. WHO 68: 323-329.

Clements, A.N. 1992. The Biology of Mosquitoes. Volume 1. Development, nutrition, and reproduction. Chapman & Hall, 509 pp.

EL-Khatib, Z.I. & Georghiou, G.P. 1985. Geographic variation of resistance to organophosphates, propoxur and DDT in the southern house mosquito, Culex quinquefasciatus, in California. J. Am. Mosq. Control Assoc. 1: 279-283.

Ferrari, J.A. & Georghiou, G.P. 1990. Esterase B1 activity variation within and among insecticide resistant, susceptible, and heterozygous strains of Culex quinquefasciatus (Diptera: Culicidae). J. Eco. Entomol. 83: 1704-1710.

Finney, D.J. 1971. Probit Analysis. Third edition. Cambridge Univ. Press., London. 333 pp.

Ford, H.R. & Green, E. 1972. Laboratory rearing of Anopheles albimanus. Mosq. News 32: 509-513.

Georghiou, G.P. 1991. New developments in biochemistry and genetics of vector resistance to pesticides and their relevance to solving control problems. CTD/OPR/EC/91. Expert committee on insecticide resistance Geneva. March 5-13, 1991; 43 pp.

Kerkut, G.A. & Gilbert, L.I. 1985. Comprehensive Insect Physiology Biochemistry and Pharmacology. Volume 12. Insect control. Pergamon Press, 849 pp.

Malcolm, C.A. 1988. Current status of pyrethroid resistance in anophelines. Parasitol. Today 4: S13-S15.

Mouches, C., Pauplin, Y., Agarwal, M., Lemieux, L., Herzog, M., Abadon, M., Beyssat-Arnaouty, V., Hyrien, O., deSaint Vincent, B.R., Georghiou, G.P. & Pasteur, N. 1990. Characterization of amplification care and esterase B1 gene responsible for insecticide resistance in Culex. Proc. Natl. Acad. Sci. USA 87: 2574-2578.

Omar, S.M., Georghiou, G.P. & Irving, N. 1980. DDT-pyrethroid resistance interrelationships in Anopheles stephensi. Mosq. News 40: 200-209.

Pasteur, N., Pasteur, G., Bonhomme, F. & Catalan, J. 1988. Practical Isozyme Genetics. Ellis Horwood Limited. 215 pp.

Peiris, H.T.R & Hemingway, J. 1990. Temephos resistance and the associated cross-resistance spectrum in a strain of Culex quinquefasciatus (Diptera: Culicidae) from Peliyagoda, Sri Lanka. Bull. Entomol. Res. 80: 49-55.

Prasittisuk, C. & Busvine, J.R. 1977. DDT resistant mosquito strains with cross-resistance to pyrethroids. Pest. Sci. 8: 527-533.

Riskallah, M.R. 1983. Esterases and resistance to synthetic pyrethroids in the Egyptian cotton leafworm. Pest. Biochem. Physiol. 19: 184-189.

World Health Organization 1975. Manual on practical entomology in malaria. Part II. Method and techniques. Geneva, WHO Offset Publication, No 13.

World Health Organization 1981a. Instructions for determining the susceptibility or resistance of mosquitoes larvae to insecticide. WHO/VBC/81.807, Geneva, Switzerland, 6 pp.

World Health Organization 1981b. Instructions for determining the susceptibility of mosquito larvae to insect development inhibitor. WHO/VBC/81.812, Geneva, Switzerland, 5 pp.

World Health Organization 1981c. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate, and carbamate insecticide-Establishment the base-line. WHO/VBC/81.805, Geneva, Switzerland, 7 pp.

World Health Organization 1981d. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate, and carbamate insecticide-Diagnostic test. WHO/VBC/81.806, Geneva, Switzerland, 6 pp.

World Health Organization 1991. Biochemical methods of detecting mechanisms of resistance and their possible role in resistance detection and monitoring by William G. Brogdon. Draft agenda item 5.2. CTD/OPR/EC/91.37, Geneva, Switzerland, 5 pp.

World Health Organization 1992. Vector resistance to pesticides. Fifteenth report of the WHO Expert Committee on vector biology and control. WHO Tech. Rept. Ser. 818, 62 pp.

TABLE 1

Percent mortality (n=3) of adult An. albimanus at the single diagnostic dosage (World Health Organization susceptibility test, 1975).

Population/ Colony**	Permethrin (0.25%)	Deltamethrin (0.025%)	DDT (4.00%)
ST	100	100	100
ES	50	50	45
TO	100	100	65
CO	100	100	100

**ST = Santa Tecla
ES = El Semillero
TO = Toledo
CO = Colozal

TABLE 2

Dose response data for permethrin, deltamethrin and DDT against An. albimanus, ST (Santa Tecla) colony, larvae.

Insecticides	LC50 (ppm)	Fiducial limits ($P < 0.05$)	LC90 (ppm)	Fiducial limits ($P < 0.05$)	Slope
Permethrin	0.0316 (0.0165)	0.0251-0.0397	0.2144 (0.1213)	0.1155-0.3202	1.5409 (0.1132)
Deltamethrin	0.0006 (0.0003)	0.0005-0.0007	0.0030 (0.0016)	0.0024-0.0041	1.9711 (0.1715)
DDT	0.00009 (0.00002)	0.00008-0.0001	0.0002 (0.0001)	0.0002-0.0003	2.9827 (0.2200)

(Standard error in parenthesis)

TABLE 3

Dose response data for permethrin and deltamethrin against An. albimanus, ES (El Semillero) colony, larvae.

Insecticides	LC50 (ppm)	Fiducial limits ($P < 0.05$)	LC90 (ppm)	Fiducial limits ($P < 0.05$)	Slope
Permethrin	0.0605 (0.0315)	0.0462-0.0788	0.7019 (0.4132)	0.4781-1.1417	1.2045 (0.0912)
Deltamethrin	0.0013 (0.0007)	0.0011-0.0015	0.0075 (0.0041)	0.0058-0.0104	1.6958 (0.1285)

(Standard error in parenthesis)

TABLE 4

Dose response data for permethrin, deltamethrin, and DDT against An. albimanus, ST (Santa Tecla) colony, adults.

Insecticides	LD50 (g/m^2)	Fiducial limits ($P < 0.05$)	LD90 (g/m^2)	Fiducial limits ($P < 0.05$)	Slope
Permethrin	0.0092 (0.0048)	0.0082-0.0108	0.0462 (0.0222)	0.0340-0.0532	1.9963 (0.1241)
Deltamethrin	0.0003 (0.0001)	0.0003-0.0004	0.0019 (0.0008)	0.0012-0.0020	2.1558 (0.1815)
DDT	0.4069 (0.2084)	0.3734-0.4434	0.7593 (0.3989)	0.67446-0.8849	4.7306 (0.3955)

(Standard error in parenthesis)

TABLE 5

Dose response data for permethrin and deltamethrin against An. albimanus, ES (El Semillero) colony, adults.

Insecticides	LD50 (g/m^2)	Fiducial limits ($P < 0.05$)	LD90 (g/m^2)	Fiducial limits ($P < 0.05$)	Slope
Permethrin	0.0189 (0.0097)	0.0166-0.0216	0.0766 (0.0411)	0.0622-0.0992	2.1099 (0.1350)
Deltamethrin	0.0007 (0.0004)	0.0006-0.0008	0.0039 (0.0021)	0.0031-0.0052	1.7395 (0.1292)

(Standard error in parenthesis)

TABLE 6

Specific activity of esterase for hydrolysis of alpha and beta naphthyl-propionate in An. albimanus adults.

Population or colony**	mg protein/ml (per mosquito)	m-mole-alpha-naphthol/min/mg protein	m-mole-beta-naphthol/min/mg protein	n
ST	0.554±0.079a*	0.585±0.184c	0.711±0.198e	59
ES	0.558±0.093a	2.755±0.084d	2.382±0.427f	60
CA	0.413±0.071b	0.437±0.161c	0.588±0.159e	72
TO	0.435±0.076b	0.399±0.105c	0.607±0.187e	32
CO	0.436±0.068b	0.383±0.114c	0.623±0.158e	44

*; Results of a and b test for protein, c and d for hydrolysis alpha naphthyl propionate, and e and f for beta naphthyl propionate.

No significant difference at 0.05 level in the same letter (within the column).

**ST = Santa Tecla
ES = El Semillero
CA = Cayo
TO = Toledo
CO = Corozal

TABLE 7

Specific activity of esterase for hydrolysis of alpha and beta naphthy-propionate in male and female of An. albimanus adults.

Population or colony**	mg protein/ml (per mosquito)	m-mole-alpha-naphthol/min/mg protein	m-mol-beta-naphthol/min/mg protein	n
ST				
Female	0.596±0.069*	0.619±0.183	0.634±0.180	36
Male	0.488±0.039	0.532±0.178	0.596±0.228	23
ES				
Female	0.598±0.100*	3.188±0.739*	2.530±0.485*	35
Male	0.522±0.043	2.028±0.354	2.173±0.042	25
CA				
Female	0.415±0.068	0.471±0.172	0.612±0.186	35
Male	0.410±0.073	0.422±0.148	0.564±0.124	37

* significant difference between male and female at 0.05 level.

**ST = Santa Tecla

ES = El Semillero

CA = Cayo

CHAPTER 3

PESTICIDE AVOIDANCE BEHAVIOR IN ANOPHELES ALBIMANUS

ABSTRACT

The behavioral responses of Anopheles albimanus Wiedemann females to DDT, permethrin, and deltamethrin were characterized. One test population (Santa Tecla colony) from El Salvador has been maintained in laboratory for 20 years. A second test population (El Semillero colony) came from Guatemala and was colonized in 1992. A third population consisted of field-caught specimens from Toledo District of southern Belize in 1994. The last population consisted of field-caught specimens from Corozal District of northern Belize in 1995. The "wild caught" populations were tested in the field. A full test compared the escape responses of test populations from each of four chambers; one that afforded direct contact with insecticide treated surfaces, one that excluded direct contact with treated surfaces, and two control chambers (one with and one without direct contact with surfaces) treated with the carrier (Risella oil) only. A very dramatic escape response of Guatemalan and Belizean mosquitoes was elicited in test chambers affording direct contact with treated surfaces for all three chemicals. Females from all but Santa Tecla colony escaped in greater numbers from chambers without direct contact with treated surfaces than escaped from control chambers ($P<0.05$); but the numbers that escaped were still less than the numbers that escaped from direct contact chambers. In marked contrast, few females from the Santa Tecla colony escaped from test chambers, regardless of insecticide used. Accelerated rates of escape from non-contact chambers is indicative of non-contact repellency. In our tests, the repellent response was significant; but not pronounced in short-term exposure (30 minutes). Repellency was increasingly obvious with greater than one-hour exposure periods. We conclude that behavioral responses of malaria vectors are important in the insecticide-malaria control equation and that this aspect of vector behavior warrants further study in the laboratory and the field.

INTRODUCTION

Anopheles albimanus Wiedemann is considered a primary vector of malaria in many areas of Central and South America (Breeland, 1974; Bruce-Chwatt, 1985). Historically, malaria has been one of the most important mosquito-borne diseases in the Americas, and beginning in the 1970s, the problem worsened as the number of malaria cases in some countries of Central and South America began to increase (World Health Organization, 1994a, b & c). In general, the trend of increasing malaria correlates with a decline in numbers of houses being sprayed for malaria control (Pan American Health Organization, 1994).

DDT has been used extensively in malaria control for decades. Today, resistance of An. albimanus to DDT occurs in several countries (Brown et al., 1976; Brown, 1986). Concomitantly, An. albimanus populations in some countries have not developed resistance in spite of regular DDT use (Roberts & Andre, 1994). Behavioral avoidance of DDT has also been reported to occur in some An. albimanus populations (Rachou et al., 1963). These phenomena raise the controversial issue of avoidance behavior (sometimes referred to as "behavioral resistance"[1]) having a role in malaria prevention and in the prevention of insecticide resistance in malaria vectors. Avoidance of DDT by malaria vectors has been recorded in the presence and absence of physiological resistance (Lockwood et al., 1985) and the relationships, if any, between resistance and behavioral avoidance are still unclear. The failure to understand these relationships is due to a remarkable shortage of systematic studies on behavioral responses of vectors to insecticides. For certain, the contributions that DDT resistance and DDT-avoidance behaviors make to the growing malaria problem are unknown.

---

[1] "The term behavioral resistance can be defined as a population-based change in a species' genetics, resulting from selective pressure of insecticide use, that increases the frequency of insecticide avoidance behavior" (Roberts & Andre, 1994).

Personnel of the World Health Organization (1970) developed an excito-repellency test to study the irritant effect of insecticides on mosquitoes. The World Health Organization test consists of counting the number of flights of a mosquito exposed to insecticide inside a transparent conical exposure chambers. Unfortunately, no method for behavioral study has been fully accepted, suggesting the difficulties of excito-repellency testing, data analysis and interpretation (Roberts et al., 1984; Evans, 1993). Furthermore, most information on avoidance behavior is on contact irritancy, and practically no studies have been conducted on non-contact repellency.

As with DDT, pyrethroids also elicit behavioral responses in insects (Threlkeld, 1985). In the United States, the horn fly (Haematobia irritans) developed resistance to synthetic pyrethroids rapidly after a few years of using ear tags impregnated with persistent pyrethroids, such as permethrin and fenvalerate (Miller, 1988). Many resistance mechanisms have been identified in the hornfly, including behavioral resistance (Lockwood et al., 1985). Recently, mosquito control through use of pyrethroid impregnated bed nets and intradomicillary spraying with pyrethroids has been introduced to many countries, including some countries of Central and South America (World Health Organization, 1989a; Curtis et al., 1989; Beach et al., 1989). The continuing prospect of wide-scale pyrethroid use should be a major stimulus for extensive studies on the significance of pyrethroid avoidance behavior in New World vectors of malaria. Clearly, the role of pyrethroid irritability and repellency should be defined for important vector species before large-scale programs are initiated.

Using colonies and wild-caught populations of An. albimanus that were characterized for isozymes, esterase and insecticide susceptibilities; research was conducted with an experimental excito-repellency test system to define the irritancy and repellency effects of DDT, permethrin, and deltamethrin. The result of this research is the subject of this report. Behavioral responses were studied with and without insecticides and with and without physical contact with insecticides.

MATERIALS AND METHODS

Anopheles albimanus test populations

1. Santa Tecla colony (ST). This colony was originally collected from a stable in La Libertad village along the Pacific Coast of El Salvador, Central America in 1975. Subsequently, the colony was maintained at the US Department of Agriculture, Gainesville, Florida and finally at the Walter Reed Army Institute of Research (WRAIR), Washington D.C. (Seawright, pers comm). We obtained this colony from the WRAIR and maintained it in the insectary at the Uniformed Services University of the Health Sciences (USUHS), Bethesda, Maryland since 1993.

2. El Semillero colony (ES). This colony was originally collected from a cattle corral in El Semillero, the Pacific Coast of Guatemala in October 1992. We obtained this colony from the Laboratory of Medical Entomology Research and Training Unit (MERTU/G), Guatemala, in 1993. This colony exhibited resistance to permethrin (Cordon-Rosales, Pers. Comm.).

3. Toledo test specimens (TO). Test specimens were obtained from human-landing collections in a rice cultivation area of San Felipe Village, Toledo District, southern Belize, in September 1994. The field-caught females were susceptible to permethrin and deltamethrin but demonstrated resistance to DDT (Chapter 2).

4. Corozal test specimens (CO). Test specimens were obtained from human-landing collections in a marsh area of Chan Chen Village, Corozal District, northern Belize in February 1995. The wild-caught females were susceptible to DDT, permethrin and deltamethrin (Chapter 2).

Insecticides

The three insecticides used in behavioral tests were:

1. Permethrin [3-phenoxybenzyl (1RS, 3RS: 1RS, 3RS)-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylate] (approx. 60% trans, 40% cis isomers) (94.7% purity). This chemical was received from Roussel Uclaf Environmental Health (UK) Ltd., United Kingdom, in January 1994. Based on established LD_{50} and LD_{90} toxicities for permethrin, test papers (12x15 cm^2) were impregnated with 0.0092 and 0.0462 g of active ingredient (a.i.)/m^2.

2. Deltamethrin [(s)-d-cyanon-phenoxybenzyl (1R, 3R)-3 (2, 2-dibromovinyl)-2, 2-dimethyl-cyclopropane carboxylate] (99.7% purity). This chemical was obtained from Roussel Uclaf Environmental Health (UK) Ltd., United Kingdom, in January 1994. Based on established LD_{50} and LD_{90} toxicities for deltamethrin, test papers (12x15 cm^2) were impregnated with 0.0003 and 0.0019 g of a.i./m^2.

3. DDT (Dichloro-diphenyl-trichloroethane) (99% purity). This chemical was purchased from the Entomological Sciences Division, United States Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland, in October, 1994. Based on established LD_{50} and LD_{90} toxicities for DDT, test papers (12x15 cm^2) were impregnated with 0.4069 and 0.7593 g of a.i./m^2. Additionally, papers were impregnated at levels of DDT used in malaria control, i.e. 2 g of a.i./m^2.

Insecticide impregnated papers were received from the Entomological Sciences Division, United States Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, Maryland. All papers were treated at the rate of 2.75 ml of the insecticide solution per 180 cm^2.

Mosquito rearing

As described in previous chapter.

Behavioral Tests

The test method used in this research consisted of enclosing 25 mosquitoes in a chamber lined with insecticide treated or untreated (control) test papers. Each chamber had a single portal for mosquitoes to escape to a receiving cage. This system accommodated a screened, second chamber (inner chamber) that, when placed in the first chamber, prevented the mosquitoes from making physical contact with test papers. Under test conditions, mosquitoes were enclosed within the chamber and the only source of light came from the exit portal. A full test consisted of a pair of treatment chambers and a pair of control chambers. This arrangement provided a matrix of test conditions, as follows:

	With or without contact with test papers	
Test Papers	With	Without
With Insecticide	X	X
Without Insecticide	X	X

One treatment chamber permitted tarsal contact with insecticide treated papers, i.e., there was no inner chamber. The second treatment chamber included the inner chamber, so mosquitoes could not make contact with insecticide treated papers. Treatment chambers were equipped with test papers that were impregnated with insecticide and an oil-based carrier. Control chambers were equipped with papers that were impregnated with carrier alone. For brevity, tests with or without the inner chambers, for either treatment or control papers, will be referred to as contact trials (no inner chamber) or non contact trials (with an inner chamber).

All test components, with the exception of a rear panel, were constructed of metal so they could be chemically cleaned. Outer chambers were constructed of stainless steel and each chamber was 34 cm X 32 cm X 32 cm. The front panel was 32 cm X 32 cm and was equipped with an escape portal. The escape portal was an outward projecting funnel, 14.75 cm at its base. The funnel louvers were 14 cm long and converged, leaving a 1.50 cm wide opening (a horizontal slit) through which the mosquitoes could escape from the chamber. The back of the outer chamber was a hinged metal door that closed tightly. The outer chamber was also equipped with a second, removable rear panel. This panel fitted inside the back of the outer chamber, abutted four small flanges inside the outer chamber, and served to imprison the test population inside the test chamber. Plexiglas was used for the second rear panel so mosquitoes could be observed inside the chamber. The plexiglas panel was equipped with a large round hole (15.5 cm in diameter) sealed with dental dam. This sealed opening was used for placing mosquitoes inside and for removing mosquitoes from the chamber. This two panel approach was used to fulfill several requirements. First, the test population needed to be in darkness so individuals could orient on light filtering through the escape funnel; thus the chamber needed to be solid and non-transparent. We fulfilled this requirement by closing the rear metal door at the start of each test. Second, we needed to be able to see inside the chamber to check for dead versus live specimens, both pre- and post-testing. We also needed to be able to see inside the chamber in order to catch and remove live specimens at the end of the test. The ability to see inside the chamber was fulfilled by using transparent plexiglas for the second panel. Third, we needed self-sealing portals for placing test populations inside the chamber and for removing specimens from the chamber at the end of each test. This requirement was fulfilled by use of a dental dam seal on the 6" diameter opening in the plexiglas rear panel.

The frame of the inner chamber was constructed of 0.62 cm X 0.62 cm aluminum beams. The structure of each chamber was 28.5 cm X 28.5 cm X 29 cm and the inner surface of each was covered with metal screening. A fine mesh screen, 52 cells per inch,

was used to cover two side walls, top and bottom of the inner chamber. The inner chamber was open ended, with 0.62 cm rubber gaskets on the front and back beams. When placed in the outer chamber, the front gasket sealed small gaps between the front stainless steel panel and the inner chamber. Likewise, the rear gasket sealed gaps between the Plexiglas panel and the inner chamber. The inner screen surface was no closer than 0.62 cm from the surface of test papers and it prevented mosquitoes from making tarsal contact with the surface of test papers. The complete illustration of the experimental escape chamber is shown in Figure 1.

To place test papers in the outer chamber, the test papers were first clipped to large sheets of clean white paper. The large papers were taped together in a ribbon effect. Then, with test papers attached, the ribbon of paper was placed against the sides, top and bottom of the outer chamber. Papers were secured to the walls by pairing a small magnet on the inside with one on the outside of the wall, and securing the paper in between the two magnets. Test papers were not positioned on the front or back of the test chamber.

A full test required four separate pint cages of 25 mosquitoes (a test population) each. In approximately one minute, an oral aspirator was used to introduce a test population into each of the four test chambers. A three-minute rest period has been used to permit the mosquitoes to adjust to the test chamber conditions in other test procedures (Busvine, 1964), and a three-minute interval was also used in this test procedure. After three minutes, the escape funnel was opened to initiate the observation period. Numbers escaping from the exposure chamber into the receiving cage were recorded in one minute intervals; after five minutes of observation, each receiving cage was replaced with a clean cage. The exchange of receiving cages facilitated the accurate counting of numbers that escaped.

Tests Performed

Only An. albimanus females were used in excito-repellency tests. Each test was replicated at least three times. To fulfill the goals of this research, tests were performed to compare insecticides, different concentrations of insecticides, insecticide resistant versus insecticide susceptible populations, colony versus field-caught populations, insecticide contact versus non-contact, and short-term (30 minutes) versus long-term (four hours) exposure.

Observations on mortality of test populations were made immediately after each test was completed, i.e., the number of dead specimens inside the exposure chamber were recorded. Additionally, test specimens collected from the exposure chamber were held separately for observations on 24-hour mortalities.

A shortage of CO specimens (Corozal, Belize) resulted in no test of DDT at 2.00 g/m^2. Likewise, a shortage of TO specimens (Toledo, Belize) resulted in no tests of DDT at 0.4060 and 0.7593 g/m^2 or of deltamethrin at 0.0003 g/m^2. The ES colony (Guatemala) was lost before tests of DDT at 0.4069 and 0.7593 g/m^2 could be conducted.

We were concerned that the 30-minute exposure period, in combination with test doses based on contact exposures followed by 24-hour mortalities, were not appropriate for detecting non-contact behavioral responses. To increase the sensitivity of the test system, we conducted tests comparing non-contact treatment versus non-contact controls for a four-hour exposure period. To prevent the possibility that insecticide particles might fall from test papers on top of the exposure chamber, clean white paper was placed on top of the inner chamber for these long-term exposures. These tests were conducted with all three insecticides against the CO population.

Data analyses

We used the life table method, a survival analysis approach, to estimate mosquito escape rates (or rate of mosquitoes staying in the chambers) and then compared differences

in mosquito escape among populations, insecticides and concentrations (doses) of insecticides. With this method, we estimated the mosquito escape rate one-minute intervals. we treated mosquitoes that escaped as "deaths" and those remaining in the exposure chamber as "survivals". The time in minutes for 50% and 90% of the test population to escape was estimated with the life table method and these estimates were used as the "escape time" summary statistics (ET_{50} and ET_{90}).

For comparing patterns of escape behavior (or survival curves), we used the log-rank method, which is designed to detect differences between survival curves (in our case, mosquito escape curves) which result when the death (or escape) rate in one group is consistently higher than the corresponding rate in a second group and the ratio of these two rates is consistent over time (in survival analysis, this is also called the proportional hazard rate). With escape data, the basic idea underlying the log rank test involves examining escape observations by one-minute intervals. To test the null hypothesis, we calculated the observed escape and expected escape in each time interval. The data were analyzed by use of tabular data presenting columns for time, number observed to escape, number expected to escape and difference between observed and expected. We then combined the tabular data for each test to give an overall measure of the deviation of the observed escape values from their expected values by each one minute test interval. The log-rank method was proposed by Mantel and Haenzel (1959), it is also called the Mantel-Cox and Peto-Haenszel methods (Mantel & Haenzel, 1959).

The log-rank test has a chi-square distribution with k degrees of freedom, where k is groups-1. A statistical software package, STATA, was used for this analysis. The p-value was used to claim that mosquito escape behaviors differed significantly among or between populations, dose levels and insecticides, if the p-value was less than 0.05.

RESULTS

Escape responses of Anopheles albimanus to DDT, permethrin, and deltamethrin were determined using contact and non-contact exposure chambers. Mortalities of adult females after a 24-hour holding period, from contact and non-contact trials, are given in Tables 1 and 2. Higher mortalities were observed in contact trials compared to non-contact trials and in non-contact trials as compared to the controls. With 2.00 g/m^2 DDT in contact trials, no mortality of escaped ES and TO females was observed, while 6.8% mortality was observed in ST females. A high mortality (38.5%) of non-escaped ST mosquitoes (96% remained in the chamber) was found as compared to trials with the ES and TO mosquitoes. With permethrin in contact trials (Table 1), less than 11% mortality of the escaped ES and TO mosquitoes was observed, while almost 40% mortality of escaped ST mosquitoes was recorded. No mortality was found from trials with the CO mosquitoes. In contact trials with deltamethrin (Table 1), higher mortality was observed in trials with the ST test population as compared to the other populations tested.

Time in minutes for mosquitoes to escape from the treated chambers is given in Table 3. The escape patterns from chambers containing insecticides were determined in terms of the escape times for 50 or 90% of the populations to leave the treated chambers (ET_{50} and ET_{90}). At 2.00 g/m^2 DDT, the ES test population had an ET_{50} and ET_{90} of 8 and 19 minutes and the TO population had an ET_{50} and ET_{90} of 2 and 16 minutes, respectively. At the lower dose of permethrin, the ES, TO and CO test populations had ET_{50}s of 2, 4, and 9 minutes, respectively. The ET_{90}s for ES and CO populations was 8 and 17 minutes, respectively (no information on ET_{90} of the TO population was obtained). Responses to deltamethrin and permethrin in all populations, except the ST population, appear to be more rapid than their responses to DDT (Table 3).

Comparisons of escape probabilities within 30 minutes between contact versus control, contact versus non-contact, and non-contact versus control in four populations of

An. albimanus are given in Table 4. Significant differences in the escape probabilities were found when contact was compared to the control in all populations ($P<0.05$).

Contact versus non-contact responses of An. albimanus to 2.000 g/m^2 DDT, 0.0092 g/m^2 permethrin, 0.0462 g/m^2 permethrin, 0.0003 g/m^2 deltamethrin and 0.0019 g/m^2 deltamethrin were compared. Significant differences were observed in ES, CO and TO populations when contact and non-contact responses to synthetic pyrethroids were compared ($P<0.05$). There was no significant difference in the ST population responses in contact versus non-contact exposures at lower doses of permethrin and deltamethrin ($P>0.05$), but significant differences were found at the higher doses ($P<0.05$). Also, significant differences in the escape responses were found in all four populations when contact responses were compared to non-contact responses. Comparisons between non-contact and control exposures are also presented in Table 4.

Statistical comparisons between 2 doses (LD_{50} and LD_{90}) of DDT, permethrin, and deltamethrin in contact and non-contact trials is given in Table 5. Higher numbers of escapes were associated with higher dose levels. No statistical differences between two doses of the three chemicals in non-contact trials were observed ($P>0.05$).

Multiple comparisons of contact trials with four test populations of An. albimanus against 2.00 g/m^2 DDT, 0.0092 g/m^2 permethrin, 0.0462 g/m^2 permethrin, 0.0003 g/m^2 deltamethrin, and 0.0019 g/m^2 deltamethrin are shown in Table 6. Marked differences in escape responses were found when the ST test population was compared with the other test populations ($P<0.05$).

Multiple comparisons of non-contact trials with four populations of An. albimanus against 2.000 g/m^2 DDT, 0.0092 and 0.0462 g/m^2 permethrin, and 0.0003 and 0.0019 g/m^2 deltamethrin also are presented in Table 6. Significant differences in escape probabilities were seen for all compounds when the ST test population was compared to the ES test population ($P<0.05$).

Figures 2-5 shows the comparison of escape probabilities between contact versus non-contact and non-contact versus control trials with 4 populations of An. albimanus. Marked differences in escape probabilities were seen when all contact trials were compared to control and non-contact trials ($P<0.05$). Escape probabilities of the ES, TO, and CO test populations in non-contact trials are higher than those from control trials. Marked non-contact repellency to DDT and synthetic pyrethroids ($P<0.05$) was seen in trials with the ES test specimens.

Tests for increased levels of escape response with four-hour exposures in non-contact trials were conducted with CO populations (Figure 6). In four-hour non-contact tests, the CO test populations showed statistically significant responses compared to the controls of all insecticides and doses ($P<0.05$). No four-hour contact trials have been performed since all CO test specimens escaped from the treated chambers within a 30-minute exposure period.

DISCUSSION

Two forms of "behavioral avoidance", contact irritability and non-contact repellency, have been described (Davidson, 1953; Rawlings & Davidson, 1982). Irritability occurs when an insect is stimulated to move away from an insecticide treated surface after it has had direct physical contact with the insecticide residue. In contrast, repellency occurs when the insect detects and avoids treated surfaces without physical contact (Kilpatrick & Schoof, 1958; Muirhead-Thomson, 1960; Roberts & Andre, 1994). In this study, both contact irritability and non-contact repellency were documented to occur with An. albimanus in the presence of DDT, permethrin, and deltamethrin.

Our laboratory and field results demonstrated that adult An. albimanus females from Guatemala (ES test population) and from Belize (TO and CO test populations) showed dramatic escape responses from exposure chambers that permitted direct contact with insecticide-treated surfaces. This irritancy response was not observed in mosquitoes that had been in colony for many years, i.e., the ST test population. There was a much weaker escape response from chambers that did not permit test populations to have direct contact with treated surfaces. Regardless, the numbers escaping from non-contact test conditions were still greater than the numbers escaping from control chambers. This suggests that both contact irritancy and non-contact repellency are involved in the escape responses of An. albimanus.

Smyth & Roys (1955) and Solimen & Cutkomp (1963) reported that DDT had a specific effect on antennal chemoreceptors and on sensory hairs of tarsal segments. The effect of DDT on sensory hairs could be considered irritability (Solimen & Cutkomp, 1963). In combination, the function of antennal chemoreceptors and sensory hairs on tarsal segments results in avoidance behaviors that involve both contact irritability and non-contact repellency. The World Health Organization (1970) reported that a number of major malaria vectors show high irritability to DDT. Behavioral avoidance of DDT has been

reported in anopheline and culicine mosquitoes (Muirhead-Thomson, 1947; Wilkinson, 1951; Elliott & de Zulueta, 1975; Brown et. al., 1976; Lockwood et al., 1984; Nutsathapana et al., 1986; Suwonkerd et al., 1990; Roberts & Andre, 1994). Most studies were conducted on the avoidance behavior of mosquitoes based on contact irritancy with various concentrations of DDT. The first published study of irritancy was conducted by Kennedy (1947) who demonstrated DDT could cause mosquitoes to escape the treated area after contact. Muirhead-Thomson, (1951) subsequently reported that Anopheles gambiae Giles would immediately try to depart a DDT-treated hut after insecticide contact. The effect of such chemicals on behavior may decrease the time mosquitoes spend searching for a blood meals; thereby decreasing disease transmission potential (Hossain & Curtis, 1989; Miller, 1990).

Many synthetic pyrethroids cause mosquitoes to escape sprayed houses (Miller, 1990; Lindsay et al., 1991). Our results demonstrated that both permethrin and deltamethrin produce strong behavioral responses from An. albimanus females. Our findings are in agreement with other studies on irritancy of synthetic pyrethroids (Taylor et al., 1981; Ree & Loong, 1989a; Ree & loong, 1989b; Pell et al., 1989).

In contrast to our findings on behavioral responses of field-caught or recently colonized populations, an older colonized population (ST test population) showed little to no behavioral response to the three insecticides tested. High mortality of the ST test population was observed in contact trials with treated surfaces. After two decades of being maintained in the laboratory, the colony has lost its natural ability to respond behaviorally to the three insecticides. A similar phenomenon was seen in earlier studies with an An. albimanus Gorgas Panama laboratory population that was maintained under laboratory conditions for 20 years. The Panama mosquitoes showed excitation times, minutes to first flight following DDT exposure, that were 2 times longer than field populations (Brown, 1958). These results caution against the use of colony populations for the study of mosquito behavioral responses to insecticides.

Aedes aegypti, An. maculipennis (Kennedy, 1947) and An. quadrimaculatus (Fay & Sheppard, 1949), when activated after contact with DDT in the laboratory, were driven from a glass box lined with filter papers impregnated with DDT before picking up a lethal dose. In the field, An. gambiae was shown to escape from a DDT-treated hut with no mortality (Muirhead-Thomson, 1950). In our studies, ES, TO and CO test specimens quickly escaped the exposure chambers without receiving a lethal dose of DDT, demonstrating strong behavioral avoidance of DDT.

Time that mosquitoes spend on treated surfaces is critical since a rapid escape decreases the chance that they will receive a lethal dose of insecticide. ES, TO, and CO test specimens were able to escape unharmed from permethrin-treated chambers. Populations of An. albimanus from Guatemala (ES) and Belize (CO and TO) that escaped from deltamethrin-treated surfaces were still alive 24 hours later. Two out of 5 non-escaped TO test specimens died within 24 hours, but escaped and non-escaped ES mosquitoes showed very low mortality. Bown et al. (1987) reported that An. albimanus from Mexico departed deltamethrin treated huts with low mortality. The escape patterns of ES and TO test populations in presence of DDT were totally different from those of the ST test population and all test populations escaping from control chambers. The TO test specimens responded to DDT more quickly than the ES test specimens in terms of rate of escape (Figure 2). This may be due to differences in test conditions, i.e., laboratory versus field conditions, and age of mosquitoes at the time of testing. Roberts et al. (1984) reported that fresh fed An. darlingi females showed lower rates of escape than unfed or late fed females when exposed to DDT. Hamon & Eyraud (1961) found that older An. gambiae and An. funestus mosquitoes demonstrated less irritability than young mosquitoes. In our studies, laboratory tests were conducted with 3 to 5-day old unfed female mosquitoes, while the physiological status of field mosquitoes was unknown. Hecht et al. (1960) reported that An. albimanus is more active at higher temperatures. Tests conducted in the field were

performed at higher ambient temperatures and humidities, which are conditions that favor greater avoidance of DDT.

A gradual escape response to permethrin and deltamethrin was observed in CO test specimens compared to ES and TO test specimens. However, all CO test specimens eventually escaped from the contact trials. The differences in escape patterns with these populations may have been influenced by differences in physiological age or gonotrophic status of female mosquitoes, as described by Busvine (1964), or due to ambient test conditions. Some of the field mosquitoes were blood fed which may have caused delayed escape patterns.

Non-contact repellency to DDT may play a role in reducing human-vector contact, as shown by studies on An. culicifacies in India (Shalaby, 1966). In our tests, the ES and TO test populations showed a certain degree of non-contact repellency to DDT, even though the exposure period was only 30 minutes. This suggests that the repellency effect of DDT is also involved in escape responses (Roberts & Andre, 1994). In short-term exposures, no repellency to synthetic pyrethroids was seen in TO test populations since numbers escaping from the treated chambers were relatively low and similar to the controls.

In contact trials, more mosquitoes escaped at higher concentrations of insecticides. At lower concentrations, synthetic pyrethroids produced poor escape responses by ST test specimens. Ree & Loong (1989a) found that An. maculatus showed an increased irritability response with increasing concentrations of permethrin. Brown (1958) found that time to first flight of An. albimanus after exposure to DDT was short at higher concentrations compared to lower concentrations. In our short-term exposures in non-contact trials, dose levels appeared to have no effect on escape patterns. As seen in the ES, TO, and CO test specimens, irritancy contributes to a strong and immediate response, while short-term exposure in non-contact trials produced a weak, but statistically significant escape response. Both irritancy and repellency are presumably additive properties producing an overall strong avoidance response.

Results from long term exposure (4 hours) to each of the three insecticides suggest that non-contact repellency is an important behavioral response in An. albimanus. Overall, deltamethrin was the most repellent, followed by permethrin and DDT. Unlike our finding with short-term exposures in non-contact trials, greater escape activity was seen at higher versus lower doses for all three compounds in four-hour exposures.

Busvine (1964) reported that the degree of irritability in mosquitoes varies by type of insecticide. Both DDT and pyrethroids generally cause mosquitoes to leave the treated surfaces before being knocked down. However, pyrethroids produce a more immediate irritant effect than DDT. Consequently, the escape times (ET_{50}s and ET_{90}s) for deltamethrin and permethrin were shorter than those for DDT. We propose that mosquitoes entering houses with insecticide treated surfaces often escape before feeding upon humans. Consequently, it is probable that a strong avoidance behavior will reduce human-vector contact and, as a result, decrease disease transmission.

Our study showed that DDT, permethrin, and deltamethrin irritate and repel An. albimanus, and most specimens that escape insecticide exposure will survive. Our findings are in agreement with Roberts & Alecrim (1991) who reported that DDT exhibits a strong repellent action. A repellent action that exerts an area affect would theoretically provide significant protection from indoor transmission of malaria. However, others propose that the irritant properties of permethrin and deltamethrin in treated huts have an unsatisfactory impact on malaria vectors (Rishikesh et al., 1978). This reasoning led to the termination of DDT use in many countries in Soviet Central Asia, Asia, and South Africa due to high irritancy (Bondareva et al., 1986; Sharp et al., 1990). Unfortunately, the use of this chemical may have been stopped for the very reason it has proven to be so effective in malaria control, viz., strong excito-repellency action. Our test results suggest that the behavioral responses elicited by either DDT, permethrin or deltamethrin might interrupt indoor An. albimanus human contact; but additional field work is needed to evaluate the impact of excito-repellent insecticides on mosquito density and malaria transmission.

No method of analysis of excito-repellency has been fully accepted or developed (Roberts et al., 1984; Evans, 1993). In this study, we used survival analysis techniques for treatment of the data. The power of the analysis is provided by using the probability of escape over time for comparing the responses of different test populations. The escaped mosquito was treated as "deaths" while the non-escaped mosquito was classified as "survivals". We believe the application of survival analysis minimizes the loss of valuable information and is the method of choice for a biological interpretation of excito-repellency test results.

To facilitate the study of behavioral responses of malaria vectors to insecticides, a portable excito-repellency test box was constructed. This box is analogous to a single house where mosquitoes are able to exit via a single outlet. The test chamber was designed for both contact and non-contact tests. The test chambers are inexpensive and can be used to test insecticides against mosquitoes accurately and quickly. However, to avoid transportation problems, a standard metal collapsible, excito-repellency test chamber should be developed.

We conclude that behavioral responses of malaria vectors to insecticides are important components of the insecticide-malaria control equation, and that these responses are often overlooked in vector control programs. More field research is needed on the behavioral responses of vector populations from different geographic locations to various insecticides. The development of standardized assays to provide indications of how an insecticide will affect vector behavior and disease transmission rates should be a subject of ongoing research.

REFERENCES CITED

Beach, R.F., Cordon-Rosales, C. & Brogdon, W.G. 1989. Detoxifying esterases may limit the use of pyrethroids for malaria control in the Americas. Parasitol. Today 5: 326-327.

Breeland, S.G. 1974. Population patterns of Anopheles albimanus and their significance to malaria abatement. Bull. WHO 50: 307-315.

Bondareva, N.I., Artem'ev, M.M. & Gracheva, G.V. 1986. Susceptibility and irritability caused by insecticides to malaria mosquitoes in USSR. Part 1. Anopheles pulcherrimus. Medit. Parazitol. Parazit. Bolezni 6: 52-55.

Bown, A.N., Frederickson, E.C., del Cananas, G.A. & Mendez, J.E. 1987. An evaluation of bendiocarb and deltamethrin applications in the same Mexican village and their impact on populations of Anopheles albimanus. PAHO Bull. 21: 121-135.

Brown, A.W.A. 1958. Laboratory studies on the behaviouristic resistance of Anopheles albimanus in Panama. Bull. WHO 19: 1053-1061.

Brown, A.W.A. 1986. Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Control Assoc. 2: 123-140.

Brown, A.W.A., Haworth, J. & Zahar, A.R. 1976. Malaria eradication and control from a global standpoint. J. Med. Entomol. 13: 1-25.

Bruce-Chwatt, L.J. 1985. Essential Malariology. Second edition. Alden Press Oxford. 452 pp.

Busvine, J.R. 1964. The significance of DDT-irritability tests on mosquitoes. Bull. WHO 31: 645-656.

Curtis, C.F., Lines, J.D., Carnevale, P., Robert, V., Boudin, C., Halna, J.M., Pazart, L., Gazin, P., Richard, A., Mouchet, J., Charlwood, J.D., Graves, P.M., Hossain, M.I., Kurihara, T., Ichimori, K., Zuzi, L., Baolin, L., Majori, G., Sabatinelli, G., Coluzzi, M., Njunwa, K.J., Wilkes, T.J., Snow, R.W. & Lindsay, S.W. 1989.

Impregnated bednets and curtains against malaria mosquitoes. pp. 5-46 in Curtis, C.F. (Ed.) Appropriate Technology for Vector Control. Boca Raton, C.R.C.

Davidson, G. 1953. Experiments on the effect of residual insecticides in houses against Anopheles gambiae and Anopheles funestus. Bull. Entomol. Res. 44: 231-254.

Elliott, R. & de Zulueta, J. 1975. Ethological resistance in malaria vectors. Behavioral responses to intradomestic residual insecticides. WHO/VBC (75.569): 15 pp.

Evans, R.G. 1993. Laboratory evaluation of the irritancy of bendiocarb, lambda-cyhalothrin and DDT to Anopheles gambiae. J. Am. Mosq. Control Assoc. 9: 285-293.

Fay, R.W. & Sheppard, E.H. 1949. Anopheles quadrimaculatus activity patterns in the laboratory on untreated and DDT-treated surfaces. J. Nat. Malar. Soc. 8: 147-158.

Hamon, J. & Eyraud, M. 1961. Study of physiological factors conditioning in Anopheles irritability to DDT. Riv. Malar. 40: 219-640.

Hecht, O., Mancera, O. & Barrera, A. 1960. Relation of DDT irritation threshold to knockdown of three species of anopheline mosquitoes. J. Eco. Entomol. 53: 634-640.

Hossain, M.I. & Curtis, C.F. 1989. Permethrin-impregnated bednets: behavioral and killing effects on mosquitoes. Med. Vet. Entomol. 3: 367-376.

Kilpatrick, J.W. & Schoof, H.F. 1958. A field strain of malathion resistant house flies. J. Econ. Entomol. 51: 18-19.

Kennedy, J.S. 1947. The excitant and repellent effects on mosquitoes of sub-lethal contacts with DDT. Bull. Entomol. Res. 37: 593-607.

Lindsay, S.W., Adiamah, J.H., Miller, J.E. & Armstrong, J.R.M. 1991. Pyrethroid-treated bednet effects on mosquitoes of the Anopheles gambiae complex in The Gambia. Med. Vet. Entomol. 5: 477-483.

Lockwood, J.A., Sparks, T.C. & Story, R.N.. 1984. Evolution of insect resistance to insecticides: A reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30: 41-51.

Lockwood, J.A., Byford, R.L., Story, R.N., Sparks, T.C. & Quisenberry, S.S. 1985. Behavioral resistance to pyrethroids in the horn fly, Haematobia irritans (Diptera: Muscidae). Environ. Entomol. 14: 873-880.

Mantel, N. & Haenzel, W. 1959. Statistical aspects of the analysis of data from retrospective studies of diseases. J. Nat. Cancer Inst. 22: 719-748.

Miller, T.A. 1988. Mechanisms of resistance to pyrethroid insecticides. Parasitol. Today 7: S8-S12.

Miller, J.E. 1990. Laboratory and field studies of insecticide impregnated fibers for mosquito control. Ph.D. dissertation, University of London. 336 pp.

Muirhead-Thomson, R.C. 1947. The effect of house spraying with pyrethrum and with DDT on Anopheles gambiae and Anopheles melas in West Africa. Bull. Entomol. Res. 38: 449.

Muirhead-Thomson, R.C. 1950. DDT and gammexone as residual insecticides against Anopheles gambiae in Africa. Trans. R. Soc. Trop. Med. Hyg. 43: 401-412.

Muirhead-Thomson, R.C. 1951. Mosquito Behavior in Relation to Malaria Transmission and Control in the Tropics. Edward Arnold & Co., London. 219 pp.

Muirhead-Thomson, R.C. 1960. The significance of irritability behavioristic avoidance and allied phenomena in malaria eradication. Bull. WHO 22: 721-731.

Nutsathapana, S., Sawasdiwongphorn, P., Chiprarop, U. & Cullen, J.R. 1986. The behavior of Anopheles minimus (Diptera: Culicidae) subjected to differing levels of DDT selection pressure in the northern Thailand. Bull. Entomol. Res. 76: 303-312.

Pan American Health Organization, 1994. Status of malaria programs in the America in the Americas. XLII Report. Pan American Health Organization, Washington, D.C.

Pell, J.K., Spinney, M.A. & Ward, K.J. 1989. Observations on the behavior of adult Anopheles gambiae encountering residual deposits of lambda-cyhalothrin compared with the other major classes. Fourth Annu. Conf. Soc. Vector Ecol. Eur. Region. 18 pp.

Rachou, R.G., Moura-Lima, M., Duret, J.P. & Kerr, J.A. 1963. Experiences with the

excito-repellency test box--model OPS. Proc. of 50th Annu Meet of N. J. Mosq. Exter. Assoc. and the 19th Ann. Meet of Am. Mosq. Control Assoc. 442-447.

Rawlings, P. & Davidson, G. 1982. The dispersal and survival of Anopheles culicifacies in Sri Lankan village under malathion spraying. Bull. Entomol. Res. 72: 139-144.

Ree, H.I. & Loong, K.P. 1989. Irritability of Anopheles farauti, Anopheles maculatus, and Culex quinquefasciatus to permethrin. Jpn. J. Sanit. Zool. 40: 47-51.

Ree, H.I. & Loong, K.P. 1989. Irritability of Anopheles pharoensis to different insecticides as observed in laboratory experiments. Sudan Med. J. 5: 18-28.

Rishikesh, N., Clarke, J.L., Mathis, H.L., King, J.S. & Pearson, J. 1978. Evaluation of decamethrin and permethrin against Anopheles gambiae and Anopheles funestus in a village trial in Nigeria. WHO/VBC/78.689.

Roberts, D.R., Alecrim, W.D., Tavares, A.M. & Mc Neill, K.M. 1984. Influence of physiological condition on the behavioral response of Anopheles darlingi to DDT. Mosq. News 44: 357-361.

Roberts, D.R. & Alecrim, 1991. Behavioral response of Anopheles darlingi to DDT sprayed house walls in Amazonia. PAHO Bull. 25: 210-217.

Roberts, D.R. & Andre, R.G. 1994. Insecticide resistance issues in vectors. Am. J. Trop. Med. Hyg. 50 (suppl): 21-34.

Shalaby, A.M. 1966. Observations on some responses of Anopheles culicifacies to DDT in experimental huts in Gujarat state, India. Ann. Entomol. Soc. Am. 59: 938-944.

Sharp, B.L., le Sueur, D. & Bekker, P. 1990. Effect of DDT on survival and blood feeding success of Anopheles arabiensis in Northern Kwazula, Republic of South Africa. J. Am. Mosq. Control. Assoc. 6: 197-202.

Smyth, T., Jr. & Roys, C.C. 1955. Chemoreceptor in insects and the action of DDT. Biol. Bull. 108: 66-76.

Soliman, S.A. & Cutkomp, L.K. 1963. A comparison of chemoreceptor and whole-fly responses to DDT and parathion. J. Econ. Entomol. 56: 492-494.

Suwonkerd, W., Amg-Ung, B., Rimwangtrakul, S., Wongkattiyakul, S., Kattiyamongkool, P., Chitprarop, V. & Takagi, M. 1990. A field study on the response of Anopheles dirus to DDT and fenitrothion sprayed to huts in Patchaboon Province, Thailand. Trop. Med. 32: 1-5.

Taylor, R.N., Hill, M.H., Stewart, D.C. & Slatter, R. 1981. A field evaluation of permethrin (OMS 1821) and BRDC 161 (OMS 1998) for residual control of mosquitoes. Mosq. News 41: 423-433.

Threlkeld, S.F.H., 1985. Behavioral responses in Drosophila melanogaster associated with permethrin and ectiban. In: Proceedings of the Thirty-Second Annual Meeting, Canadian Pest Management Society, Charlottetown, Prince Island 24-26 June, 1985. Can. Pest Manage. Soc. 29-36.

Wilkinson, P.R. 1951. Distribution and fate of Anopheles gambiae and Anopheles funestus in 2 different types of huts treated with DDT and BHC in Uganda. Bull. Entomol. Res. 42: 45-54.

World Health Organization 1970. Insecticide resistance and vector control (17th report of the WHO Expert Committee on Insecticides). Instructions for determining the irritability of adult mosquitoes to insecticide. WHO Tech. Rep. Ser. 433: 158-163.

World Health Organization 1989. The use of impregnated bednet and other materials for vector-borne disease control. WHO/VBC/89.981, Geneva, Switzerland,45 pp.

World Health Organization 1994a. Weekly Epidemiological Record 42. World Health Organization Geneva, Switzerland, 309-314.

World Health Organization 1994b. Weekly Epidemiological Record 43. World Health Organization Geneva, Switzerland, 317-323.

World Health Organization 1994c. Weekly Epidemiological Record 44. World Health Organization Geneva, Switzerland, 325-330.

TABLE 1

Mortalities of An. albimanus after a 24-hour holding period in the contact trials.

Population or Colony*	Insecticide	Dose g/m^2	Tested	Number or % of specimens: Escaped (%escape)	Dead	%Mortality	Number or % of specimens: Not Escaped	Dead	%Mortality
ST colony El Salvador	Permethrin	0.0092	100	13(13)	1	7.6	87	10	11.5
		0.0000	100	0(0)	0	0	100	0	0
		0.0462	100	23(23)	9	39.1	77	45	58.4
		0.0000	100	3(3)	0	0	97	0	0
	Deltamethrin	0.0003	75	7(9.3)	0	0	68	2	3
		0.0000	75	1(1.3)	0	0	74	0	0
		0.0019	150	28(18.6)	5	17.8	122	33	27
		0.0000	150	1(0.6)	0	0	149	0	0
	DDT	2.0000	125	29(23.2)	2	6.8	96	37	38.5
		0.0000	125	7(5.6)	0	0	118	0	0
ES colony Guatemala	Permethrin	0.0092	150	150(100)	13	8.6	0	0	0
		0.0000	150	9(6)	0	0	141	0	0
		0.0462	100	97(97)	11	11	3	0	0
		0.0000	100	0(0)	0	0	100	0	0
	Deltamethrin	0.0003	75	75(100)	2	2.6	0	0	0
		0.0000	75	6(8)	0	0	69	0	0
		0.0019	75	71(94.5)	4	5.6	4	0	0
		0.0000	75	3(4)	0	0	72	0	0
	DDT	2.0000	75	72(96)	0	0	3	0	0
		0.0000	75	4(5.3)	0	0	71	0	0
TO population Belize	Permethrin	0.0092	76	67(88.1)	2	2.9	9	4	44.4
		0.0000	76	8(10.5)	0	0	68	0	0
		0.0462	90	90(100)	2	2.2	0	0	0
		0.0000	75	4(5.3)	0	0	71	0	0
	Deltamethrin	0.0019	75	70(93.3)	1	1.42	5	2	40
		0.0000	75	4(5.3)	0	0	71	0	0
	DDT	2.0000	115	113(98.2)	0	0	2	1	50
		0.0000	100	17(17)	0	0	83	0	0
CO population Belize	Permethrin	0.0092	75	75(100)	0	0	0	0	0
		0.0000	75	3(4)	0	0	72	0	0
		0.0462	75	74(98.7)	0	0	1	0	0
		0.0000	75	6(8)	0	0	69	0	0
	Deltamethrin	0.0003	75	75(100)	0	0	0	0	0
		0.0000	75	7(9.3)	0	0	68	0	0
		0.0019	75	75(100)	0	0	0	0	0
		0.0000	75	2(2.7)	0	0	73	0	0
	DDT	0.4069	75	71(94.7)	0	0	4	1	25
		0.0000	75	2(2.7)	0	0	73	0	0
		0.7593	75	70(93.3)	1	1.42	5	1	20
		0.0000	75	8(10.7)	0	0	67	0	0

* ST: Santa Tecla, ES: El Semillero, TO: Toledo: CO: Corozal

TABLE 2

Mortalities of An. albimanus after a 24 hour-holding period in the noncontact trials.

Population or colony	Insecticide	Dose g/m^2	Tested	Number or % of specimens			Number or % of specimens		
				Escapee(% escape)	Dead	%Mortality	Not Escaped	Dead	%Mortality
ST colony El Salvador	Permethrin	0.0092	100	4(4)	0	0	96	0	0
		0.0000	100	1(1)	0	0	99	0	0
		0.0462	100	1(1)	0	0	99	0	0
		0.0000	100	3(3)	0	0	97	0	0
	Deltamethrin	0.0003	75	2(2.6)	0	0	73	0	0
		0.0000	75	3(4)	0	0	72	0	0
		0.0019	150	3(2)	0	0	147	0	0
		0.0000	150	0	0	0	150	0	0
	DDT	2.0000	125	4(3.2)	0	0	121	0	0
		0.0000	125	5(4)	0	0	120	0	0
ES colony Guatemala	Permethrin	0.0092	125	24(19.3)	1	4.1	101	1	1
		0.0000	125	4(3.2)	0	0	121	0	0
		0.0462	75	8(10.7)	0	0	67	1	1.5
		0.0000	75	2(2.6)	0	0	73	0	0
	Deltamethrin	0.0003	75	14(18.7)	0	0	61	0	0
		0.0000	75	3(4)	0	0	72	0	0
		0.0019	75	15(20)	0	0	60	0	0
		0.0000	75	4(5.3)	0	0	71	0	0
	DDT	2.0000	75	18(24)	0	0	57	0	0
		0.0000	75	6(8)	0	0	69	0	0
TO population Belize	Permethrin	0.0092	75	8(10.7)	0	0	67	0	0
		0.0000	75	7(9.3)	0	0	68	0	0
		0.0462	75	0(0)	0	0	75	0	0
		0.0000	75	2(2.6)	0	0	73	0	0
	Deltamethrin	0.0019	75	8(10.7)	0	0	67	0	0
		0.0000	75	5(6.6)	0	0	72	0	0
	DDT	2.0000	100	24(24)	0	0	76	0	0
		0.0000	100	6(6)	0	0	94	0	0
CO population Belize	Permethrin	0.0092	75	6(8)	0	0	69	0	0
		0.0000	75	3(4)	0	0	72	0	0
		0.0462	75	6(8)	0	0	69	0	0
		0.0000	75	4(5.3)	0	0	71	0	0
	Deltamethrin	0.0003	75	10(13.3)	0	0	65	0	0
		0.0000	75	3(4)	0	0	72	0	0
		0.0019	75	7(9.3)	0	0	68	0	0
		0.0000	75	5(6.7)	0	0	70	0	0
	DDT	0.4069	75	4(5.3)	0	0	71	0	0
		0.0000	75	1(1.3)	0	0	74	0	0
		0.7593	75	6(8)	0	0	69	0	0
		0.0000	75	3(4)	0	0	72	0	0

* ST: Santa Tecla, ES: El Semillero, TO: Toledo: CO: Corozal

TABLE 3

Time in minutes for 50 and 90% (ET_{50} and ET_{90}) for An. albimanus females to escape from exposure chambers treated with DDT, permethrin or deltamethrin.

Population/ colony**	DDT (2.00g/m2)		Permethrin (0.0092 g/m2)		Permethrin (0.0462 g/m2)		Deltamethrin (0.0003 g/m2)		Deltamethrin 0.0019 g/m2)	
	ET50	ET90	ET50	ET90	ET50	ET90	ET50	ET90	ET50	ET90
ST colony	*	*	*	*	*	*	*	*	*	*
ES colony	8	19	2	8	1	7	7	11	2	6
TO population	2	16	4	*	1	7	-	-	1	6
CO population	-	-	9	17	4	11	9	19	4	10

* can not measure within 30 minutes
\- mosquitoes are not available to the test
** ST: Santa Tecla, ES: El Semillero, TO: Toledo: CO: Corozal

TABLE 4

Comparison in responses between contact and control trials, contact and non-contact trials, and non-contact and control trials in terms of insecticide for each population of An. albimanus.

Chemical	Population/ Colony**	Contact vs control Dose (g/m2)	Contact vs non contact Dose (g/m2)	Non contact vs control Dose (g/m2)
DDT	ST	2.0000	2.0000	2.0000*
	ES	2.0000	2.0000	2.0000
	CO	0.4069	0.4069	0.4069
		0.7593	0.7593	0.7593*
	TO	2.0000	2.0000	2.0000
Permethrin	ST	0.0092	0.0092*	0.0092*
		0.0462	0.0462	0.0462*
	ES	0.0092	0.0092	0.0092
		0.0462	0.0462	0.0462
	CO	0.0092	0.0092	0.0092
		0.0462	0.0462	0.0462
	TO	0.0092	0.0092	0.0092*
		0.0462	0.0462	0.0462*
Deltamethrin	ST	0.0003	0.0003	0.0003*
		0.0019	0.0019	0.0019*
	ES	0.0003	0.0003	0.0003
		0.0019	0.0019	0.0019
	CO	0.0003	0.0003	0.0003
		0.0019	0.0019	0.0019
	TO	0.0019	0.0019	0.0019*

* No significant difference at 0.05 level
**ST: Santa Tecla, ES: El Semillero, TO: Toledo: CO: Corozal

TABLE 5

Comparison between 2 doses (LD_{50} and LD_{90}) of each insecticide with the same population of An. albimanus.

Insecticide	Status	Population/ Colony**	Compared dose level
DDT	Contact	CO	0.4069 vs 0.7593*
	Noncontact	CO	0.4069 vs 0.7593*
Permethrin	Contact	ST	0.0092 vs 0.0462*
		ES	0.0092 vs 0.0462
		CO	0.0092 vs 0.0462
		TO	0.0092 vs 0.0462
	Noncontact	ST	0.0092 vs 0.0462*
		ES	0.0092 vs 0.0462*
		CO	0.0092 vs 0.0462*
		TO	0.0092 vs 0.0462*
Deltamethrin	Contact	ST	0.0003 vs 0.0019*
		ES	0.0003 vs 0.0019*
		CO	0.0003 vs 0.0019
	Noncontact	ST	0.0003 vs 0.0019*
		ES	0.0003 vs 0.0019*
		CO	0.0003 vs 0.0019*

* No significant difference at 0.05 level

** ST: Santa Tecla, ES: El Semillero, TO: Toledo: CO: Corozal

TABLE 6

Comparison between populations of An. albimanus in contact and non-contact trials within the same dose.

Insecticide	Dose (g/m2)	Contact Population or colony**	Non contact Population or colony
DDT	2.0000	ST vs ES	ST vs ES
		ST vs TO	ST vs TO
		ES vs TO	ES vs TO*
Permethrin	0.0092	ST vs ES	ST vs ES
		ST vs CO	ST vs CO*
		ST vs TO	ST vs TO*
		ES vs CO	ES vs CO*
		ES vs TO	ES vs TO*
		CO vs TO*	CO vs TO*
Permethrin	0.0462	ST vs ES	ST vs ES
		ST vs CO	ST vs CO*
		ST vs TO	ST vs TO*
		ES vs CO*	ES vs CO
		ES vs TO	ES vs TO
		CO vs TO	CO vs TO*
Deltamethrin	0.0003	ST vs ES	ST vs ES
		ST vs CO	ST vs CO
		ES vs CO*	ES vs CO*
Deltamethrin	0.0019	ST vs ES	ST vs ES
		ST vs CO	ST vs CO
		ST vs TO	ST vs TO*
		ES vs CO*	ES vs CO
		ES vs TO	ES vs TO
		CO vs TO*	CO vs TO*

* No significant difference at 0.05 level

** ST: Santa Tecla, ES: El Semillero, TO: Toledo: CO: Corozal

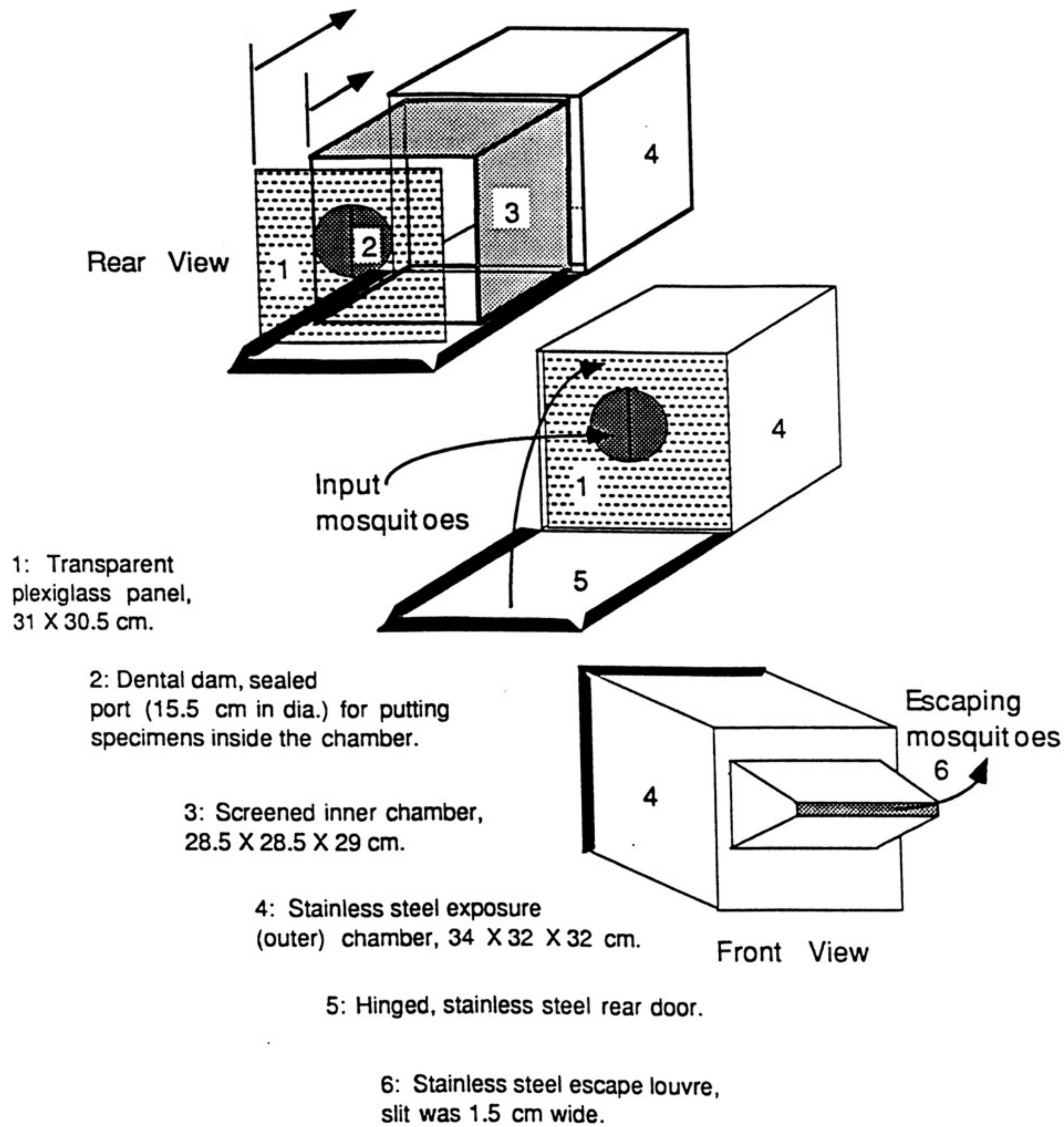


Figure 1. An excito-repellency test chamber for the study of behavioral responses of mosquitoes exposed to insecticides.

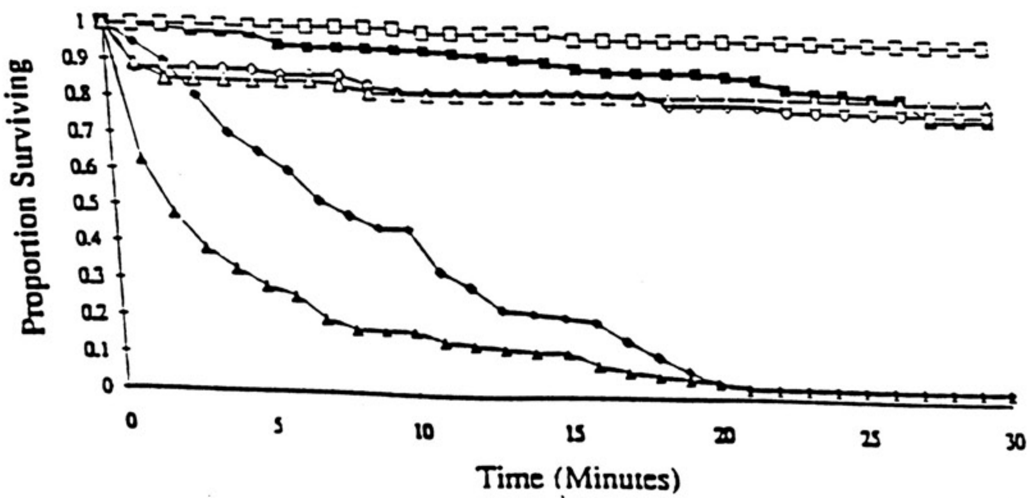


Figure 2. Comparison between contact and non-contact trials with 2.00 g/m^2 DDT against An. albimanus.

Contact ST — Non-contact ST — Contact ES

Non-contact ES — Contact TO — Non-contact TO

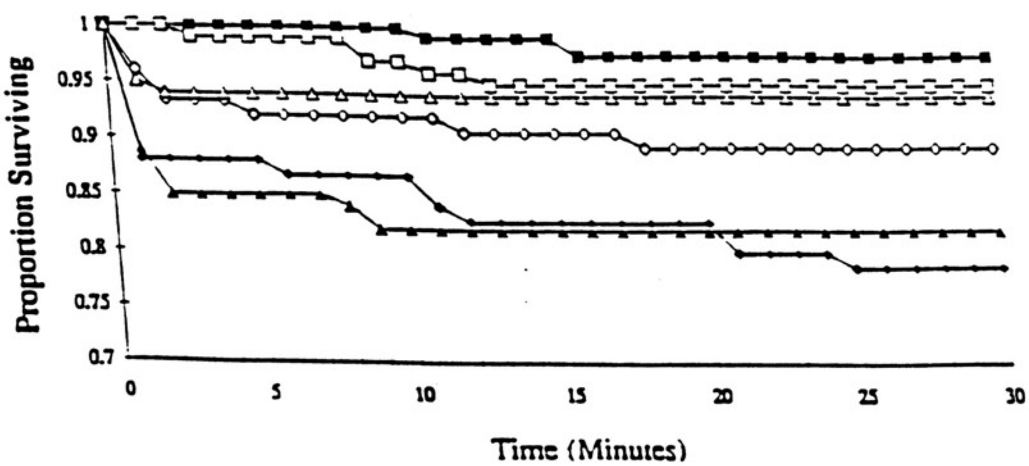


Figure 3. Comparison between non-contact and control trials with 2.00 g/m^2 DDT agains: An. albimanus.

Non-contact ST — Control ST — Non-contact ES

Control ES — Non-contact TO — Control TO

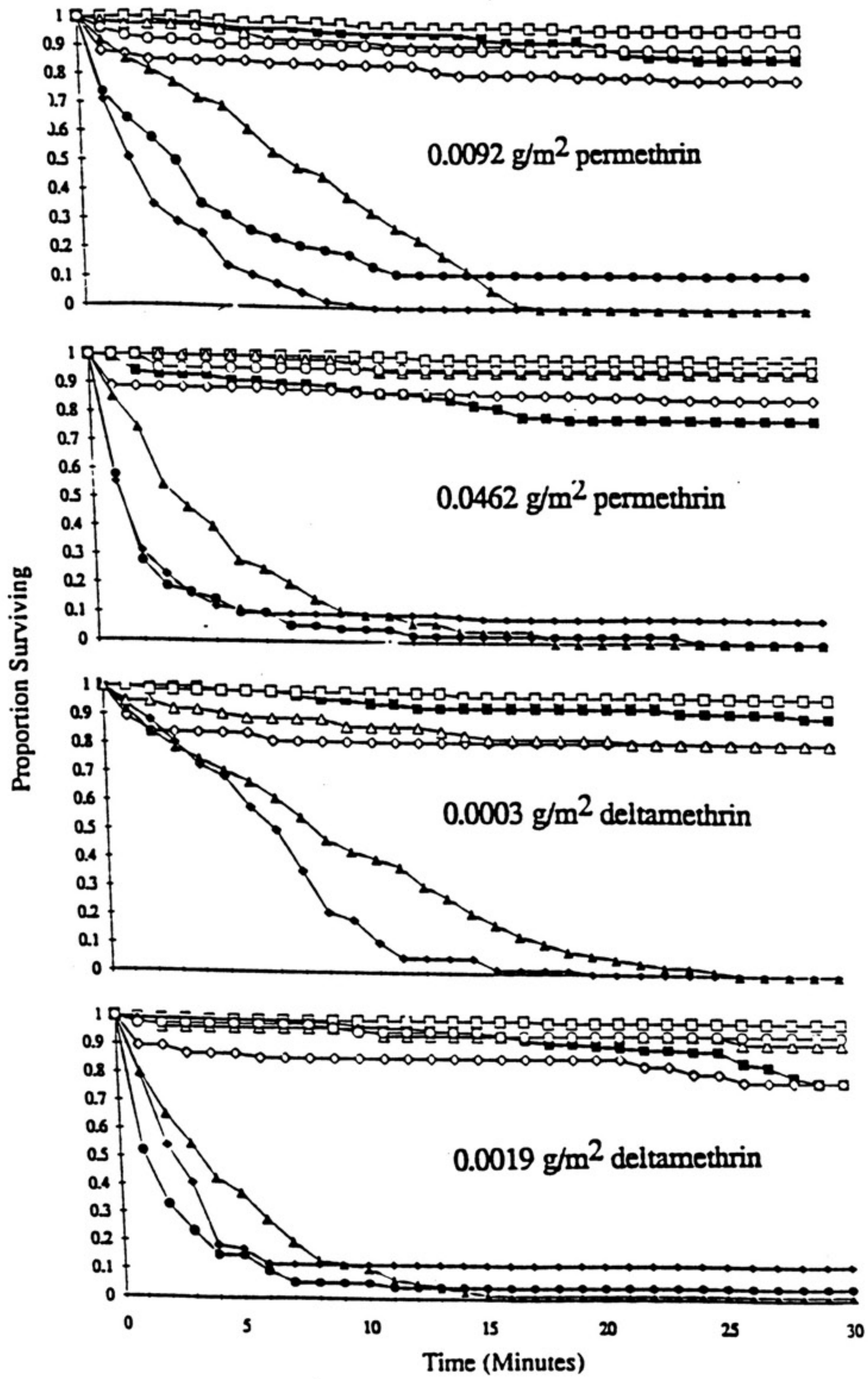
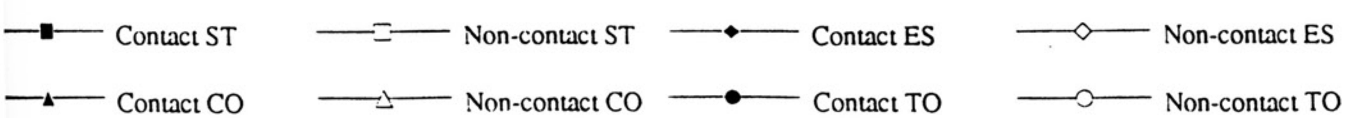


Figure 4. Comparison between contact and non-contact trials with permethrin and deltamethrin against An. albimanus.



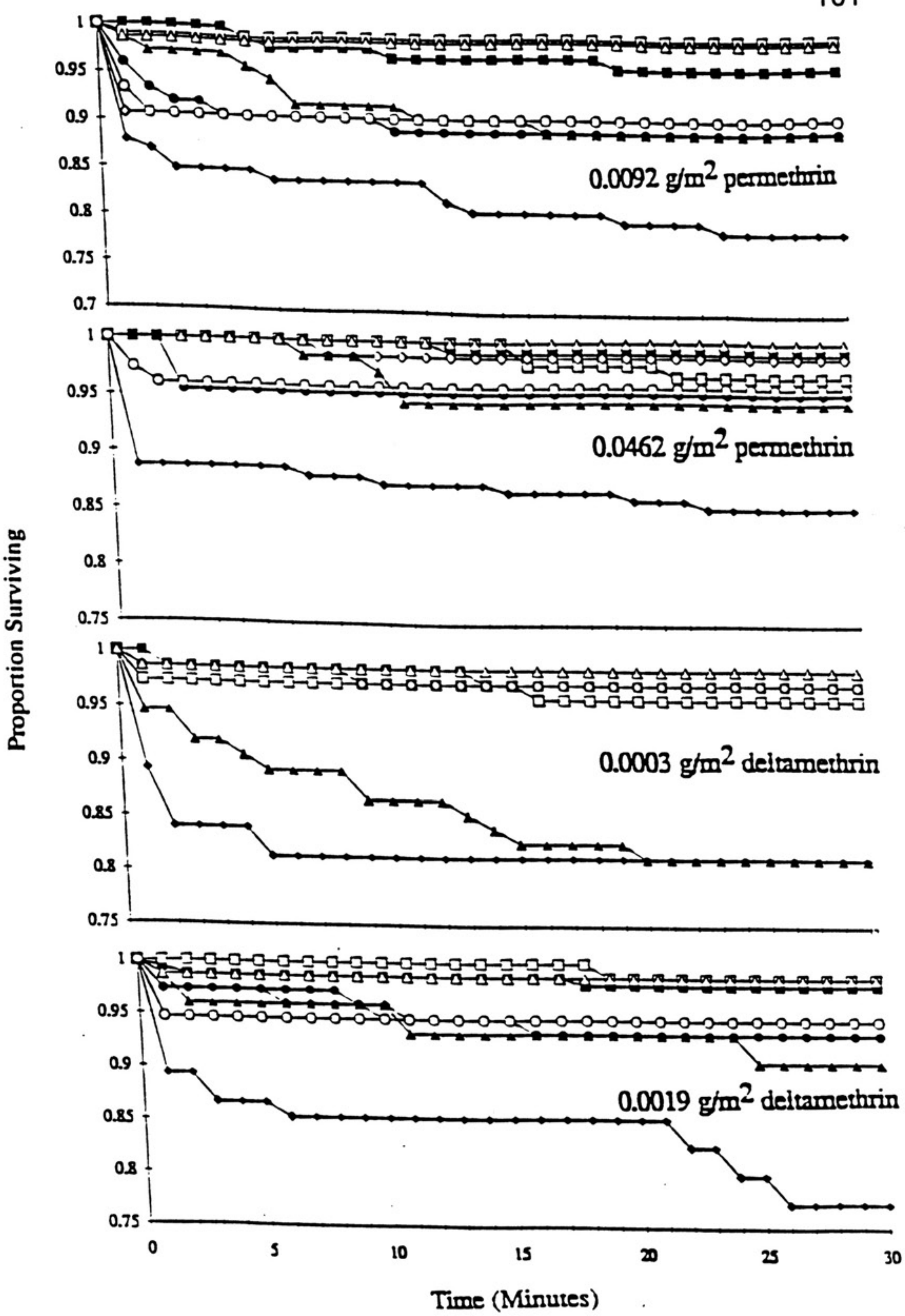
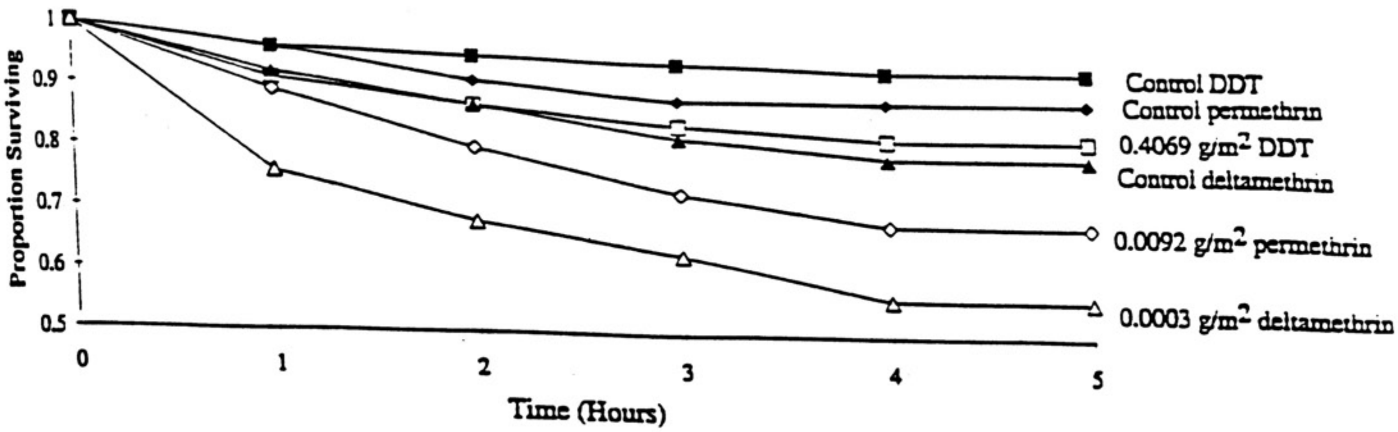


Figure 5. Comparison between non-contact and control trials with permethrin and deltamethrin against An. albimanus.

Non-contact ST — Control ST — Non-contact ES — Control ES

Non-contact CO — Control CO — Non-contact TO — Control TO



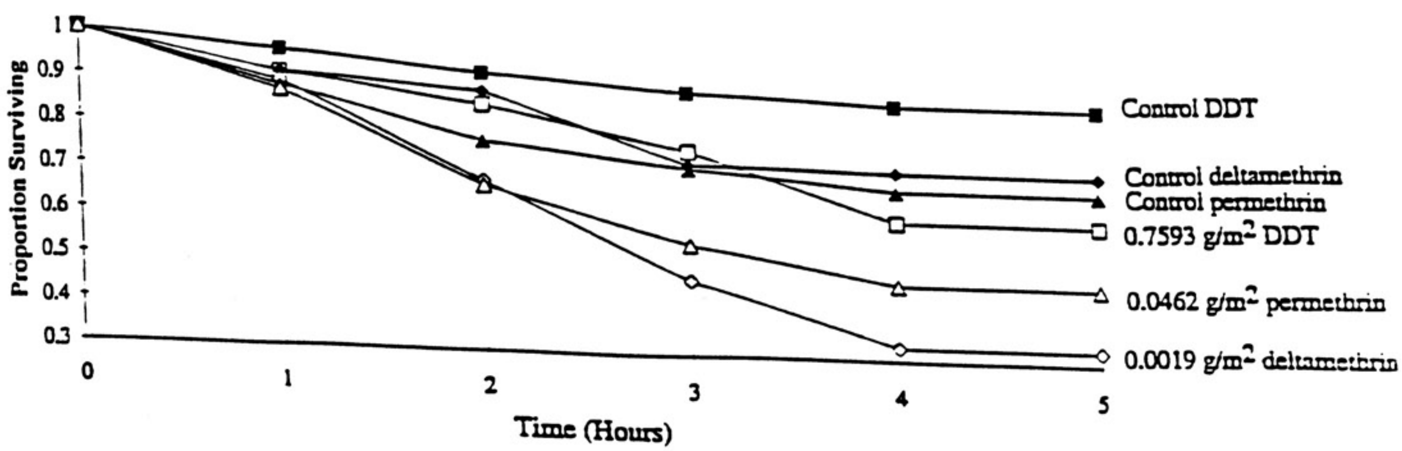


Figure 6. Comparison between non-contact and control with DDT, permethrin, and deltamethrin against An. albimanus, Corozal.

CHAPTER 4

BIOCHEMICAL SYSTEMATICS OF *ANOPHELES ALBIMANUS* WIEDEMANN AND ENZYME PATTERN IN THE PYRETHROID RESISTANT STRAIN

ABSTRACT

Isozymes of six populations of Anopheles albimanus Wiedemann were compared using starch and polyacrylamide gel electrophoresis. Tests were performed on laboratory colonies from El Salvador (Santa Tecla colony), Guatemala (El Semillero colony), and Mexico (southern Mexican colony) and three wild-caught populations from Belize (Cayo, Toledo, and Corozal populations). The Santa Tecla colony has been maintained in the laboratory for at least 20 years, but the El Semillero and southern Mexican colonies have been maintained in laboratory for only two years. From a total of 31 enzyme systems, 24 were consistently detected and 35 putative loci were scorable. Higher genetic variability was found in the three wild-caught populations from Belize and the young colonies from Guatemala and Mexico as compared to the old colony from El Salvador. Mean Heterozygosity (MH) values of wild populations from Belize and the young colonies from Guatemala and southern Mexico were calculated; values of MH ranged from 0.093 to 0.200, compared to the old colony from El Salvador with the value of 0.057. Detailed analyses showed all six populations of An. albimanus to be conspecific with minor intraspecific variation.

Zymograms were compared among six populations of An. albimanus. These included a pyrethroid-resistant colony (El Semillero colony). One locus of esterase, Est-3, and two loci of leucine-amino-peptidase, Lap-1 and Lap-2, were found to be diagnostic for separating susceptible and resistant populations. Since esterase and leucine-amino-peptidase levels were consistently elevated in resistant population, we conclude that esterase and leucine-amino-peptidase may be specifically involved in the metabolic detoxification pathway in pyrethroid resistant populations.

Due to the regular uses of organophosphate and carbamate insecticides in Guatemala, the elevated esterase in El Semillero colony may also be associated with these two compounds. Therefore, the presence of both enzymes in wild An. albimanus

populations may be related to organophosphate, carbamate, pyrethroids, or all three compounds.

INTRODUCTION

Anopheles albimanus Wiedemann (Diptera: Culicidae) is widely distributed throughout the tropics and subtropics of the Americas (Darsie & Ward, 1981) as shown in Figure 1. This species has been incriminated as an important malaria vector in Central America and northern South America (Bruce-Chwatt, 1985). Anopheles albimanus is resistant to most insecticides used in public health along the coastal areas of Central America (Brogdon et al., 1988; World Health Organization, 1992). Many insecticide resistance mechanisms have been reported in arthropods of medical importance (Roberts & Andre, 1994). Enzyme detoxification, by modifying or increasing endogenous enzymes within the insect, is a major mechanism of resistance (Georghiou & Pasteur, 1978). O-demethylase was reported to be the primary detoxification enzyme of methoprene in the house fly, Musca domestica (Hammock et al., 1977). Carboxylesterases, phosphotriesterases, acetylcholinesterases, glutathion-dependent-transferase, and mixed function oxidases are important in organophosphate resistance (Georghiou & Pasteur, 1978). Recently, Beach et al. (1989a) proposed that detoxification of pyrethroids by elevated esterases in numerous populations of An. albimanus may limit the usefulness of pyrethroids for malaria control in the Americas. Elevated esterase levels correlated well with the survival rate after exposure to synthetic pyrethroids.

Biochemical differences exist within the same detoxification enzymes between susceptible and resistant insects. Therefore, some studies on insecticide resistance have focused on electrophoretic analyses, and this technique can serve as a means of identifying resistant genotypes in mosquito populations (Georghiou & Pasteur, 1978).

Electrophoretic methods have been used since 1960 (Harris & Hopkinson, 1976) for the study of genetics and evolutionary biology of a wide range of organisms which are difficult to identify by morphological means. This technique can be used to delimit phylogenetic relationships, investigate species homologies, and study ecological-genetic

relationships. The objective of the present study was to compare isozymes of susceptible and resistant populations of adult An. albimanus and to compare genetic isozyme expression among six populations. Laboratory populations of An. albimanus from El Salvador, Guatemala, and southern Mexico, and three wild-caught populations from Belize were compared using starch and polyacrylamide gel electrophoresis (PAGE).

MATERIALS AND METHODS

Anopheles albimanus populations

1. Santa Tecla colony (ST colony): This permethrin and deltamethrin susceptible colony from El Salvador was collected from La Libertad and was originally colonized at Santa Tecla, El Salvador in 1974. We obtained the colony from the Walter Reed Army Institute of Research (WRAIR) in 1993.

2. El Semillero colony (ES colony): This permethrin and deltamethrin resistant colony was originally collected as adults from a cattle corral in El Semillero, the Pacific Coast of Guatemala in October 1992. We obtained this colony from the Laboratory of Medical Entomology Research and Training Unit (MERTU/G), Guatemala in 1993.

3. Southern Mexican Colony (SM colony): This colony has been maintained at the Centro de Investigacion de Paludismo (CIP), southern Mexico since 1986. We obtained frozen specimens in February, 1991.

4. Cayo population (CA population): A Belizean population was collected as larvae in Cayo District (Central Belize) and reared to adults in the laboratory of the Epidemiological Research Center (ERC), Belize city, Belize, Central America. Frozen specimens were brought back to the Uniformed Services University of the Health Sciences (USUHS) in May, 1994. No information on insecticide resistance is available.

5. Toledo population (TO population): A permethrin and deltamethrin susceptible Belizean population was obtained from human-landing collections in a rice cultivation area of San Felipe village, Toledo District (southern Belize) in September, 1994.

6. Corozal population (CO population): A permethrin and deltamethrin susceptible Belizean population was obtained from human-landing collections in a marsh area near Chan Chen Village, Corozal District (northern Belize) in February, 1995.

Adults from Belize (from all three populations) were identified and frozen alive in liquid nitrogen and returned to USUHS for electrophoresis study.

Mosquito rearing

The Cayo population was reared to the adult stage following the methods by Ford & Green (1972) in the laboratory of the Epidemiological Research Center, Belize city, Belize. As the specimens emerged, they were quickly killed and frozen. The Santa Tecla and El Semillero colonies were maintained in an insectary at USUHS as described in previous chapter.

Starch gel electrophoresis

Starch gel electrophoresis was performed using a standard battery of 31 enzyme-staining systems. All specimens of An. albimanus were identified and stored at -70°C until testing (Manguin et al., 1995; Harris & Hopkinson, 1976).

Six buffer systems were used for the preliminary examination of each enzyme system to determine which buffer provided the best allelic resolution: Tris-malate-EDTA buffer system (TME) (Pasteur et al., 1988), Tris-citrate buffer system (TCss) (Shaw & Prasad, 1970), Morpholine buffer system (Morph) (Pasteur et al., 1988), Lithium buffer system (LiOH) (Pasteur et al., 1988), Tris-borate-EDTA buffer system (TBE) (Pasteur et al., 1988), and Pouliks buffer system (Poulik, 1957). Of these, four buffer systems listed in Table 1 were performed on 24 enzyme systems.

The processes of horizontal gel electrophoresis were conducted as described by Harris and Hopkinson (1976). After screening, electrophoresis was carried out on horizontal starch gels using 25 g starch (Sigma), 25 g electrostarch (Electrostarch Co), 20 g sucrose, and 400 ml gel buffer. Twenty-four of 31 enzyme systems had good allelic resolution including 35 putative loci (Table 1). Each individual mosquito was ground in 25 µl of grinding buffer (25 µl/4 wicks), and the homogenate was absorbed onto 4x11 mm type wicks (cellulose polyacetate) (German Sciences Inc, Michigan). Each mosquito was run on four different buffers simultaneously. The TCss, LiOH, and Morph gels were run for six hours at a constant power of 16 volts/cm (Manguin & Hung, 1991). The TME gel

was run for 12 hours at a constant power of 8 volts/cm. Each gel was stained and incubated at 37°C for 15 to 60 minutes.

Analysis of allelic frequencies, heterozygosities, the Hardy-Weinberg equilibrium and genetic distance were calculated using BIOSYS-1 (Swofford & Selander, 1981). Each population was analyzed by the computation of heterozygosity per locus, genetic distance, and a test for conformance to Hardy-Weinberg equilibrium at single locus by a chi-square test. Differentiation among populations was determined by F-statistics. Nei's (1978) unbiased genetic distance was used for the cluster analysis by the unweighted pair-group method arithmetic averaging (UPGMA) to produce the phenogram.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was used to better resolve particular esterases in pyrethroid susceptible and resistant mosquitoes (El Semillero) that show similar mobilities on horizontal starch gel electrophoresis. Five percent polyacrylamide solution was made using 8.33 ml of 30% acrylamide stock solution, 5 ml of 10x tris-malate-EDTA (TME) electrode buffer, 0.35 ml of 10% ammonium persulfate, 17.5 μl of TEMED and 36.35 ml of distilled water to make up the total volume of 50 ml (Raymond, 1962).

Individual mosquitoes were homogenized mechanically in a plastic 1.5 microcentrifuge tube with a pestle in 50 μl grinding buffer, plus 50 μl 10% glycerol containing 0.1% of bromophenol blue loading dye. A quantity of 30 μl of each sample was loaded into each lane (18 lanes/gel) [10% glycerol included in the specimen to prevent dispersion in the buffer]. After sample loading, a voltage of 60V was applied for the first 30 minutes before increasing to 110V. The current was stopped when the bromophenol blue had reached 1 cm from the bottom of the gel (Green et al., 1990).

RESULTS

Among 35 loci (Table 2), six showed allelic polymorphism in the ST colony, 14 in the ES colony and 13 in the SM colony. For the wild-caught populations from Belize, 17 loci showed allelic polymorphism in the CA population, 19 in the TO population, and 23 in the CO population. Six loci, Ak-1, Ak-2, Gr-1, Had-1, Idh-1 and Pgm-1, showed allelic polymorphic in all test populations. Seventeen loci, Acph-1, Ak-1, Ak-2, Ao-1, Est-3, Got-1, Got-2, a-Gpdh-1, Gr-1, Had-1, Idh-1, Lap-2, Mdh-1, Me-1, 6-Pgd-1, Pgm-1, and Tpi-1, showed allelic polymorphism in all three Belize wild-caught populations. Est-1 and Lap-1 showed allelic polymorphic polymorphism only in the TO and CO populations, not in the CA population.

The chi-square test was performed between observed and expected allele frequencies. Of the 210 comparisons made, nine significant deviations were observed (Table 2, double asterisks), and these represent less than 5% of the expected deviations from the Hardy-Weinberg Equilibrium by chance only. The banding patterns from Ao-1 and Me-1 were sometimes too difficult to score and may have resulted in some minor scoring errors. The reasons for the apparent deviations seen in Got-1, Idh-1, and Pgm-1 are unknown.

The percentage of polymorphic loci (Table 3), at the 95% confidence limits, was 14.3 for the old colony (ST) whereas ranged from 28.6 to 48.6 for the recently colonized (ES and SM) and field populations (CA, TO and CO). The mean heterozygosity (direct-count) for all 35 loci varied from 0.057 for the ST colony to 0.200 for CO population. The mean number of alleles per locus was 1.2 for the ST colony, 1.5 for the ES and the SM colonies, 1.7 for the CA population, and 1.8 for the TO and the CO populations.

F- statistics, measure of the amount of differentiation among the six An. albimanus populations, F_{ST} (Wright, 1978) showed an average value of 0.243 and F_{IS} had a mean value of 0.097 (Table 4). However, the F_{IS} had a value of 0.077 when the old colony

(ST) was not included in the analysis. A large differentiation ($F_{ST} > 0.25$) (Wright, 1978) was found in four loci, Est-1 ($F_{ST} = 0.777$), Mdh-1 ($F_{ST} = 0.434$), Me-1 ($F_{ST} = 0.414$), and 6Pgd-1 ($F_{ST}= 0.530$). However, a moderate differentiation was found when all 35 loci were considered together ($F_{ST}=0.243$). In the case of Est (Table 2), the ST, ES, SM, CA and TO test populations have a high frequency (95-100%) for Est-1 (allele 115) whereas, the CO population showed a low frequency (14.9%) for Est-1 (115). For Mdh (Table 2), the ES, SM, CA, TO and CO test populations have a low to moderate frequency (0-53%) for Mdh-1 (allele 110) whereas, the ST test population showed a very high frequency (100%) for Mdh-1 (110). Mdh-1 (allele 90) was not detected in the ST, ES and TO test populations. For Me (Table 2), all An. albimanus populations have a low frequency (1.5-22.7%) for Me-1 (allele 107), except the one from the ST test population which has a very high frequency (100%) for ME-1 (107). With 6Pgd (Table 2), 6Pgd-1 (allele 100) was not found in the ST, ES and SM and 6Pgd-1 (allele 84) was not found in the ST, ES, SM and CA test populations.

The estimates of Nei's (1978) unbiased genetic identities of any two among the six populations of An. albimanus varied from 0.889 to 0.981 and the genetic distances from 0.019 to 0.117 (Table 5). Nei's (1978) unbiased genetic distance was clustered by the unweighted pair group method (UPGMA) to produce the phenogram as shown in Figure 2. Phenograms produced by using other distance measures, such as modified Rogers (Wright, 1978) and Cavalli-Sforza & Edwards (1967) showed nearly identical branching patterns. The CA and TO populations are more closely related to the SM and ES colonies than they were are to the CO population. The ST colony was markedly different from the other populations with a Nei's genetic distance averaging 0.29.

Paired analyses were conducted using an F-statistic by comparing all combinations of the six populations when all 35 loci were considered together (Table 6). Great differentiations with $F_{ST} >0.25$ were found between the ST and ES ($F_{ST} = 0.339$), ST and SM ($F_{ST} = 0.275$), ST and CA ($F_{ST} = 0.277$), and ST and CO ($F_{ST} = 0.269$) test

populations. Only moderate differentiations were found between the ST and TO (F_{ST} = 0.161), ES and CO (F_{ST} = 0.199), and SM and CO (F_{ST} = 0.157) test populations. Small differentiations were found between the ES and CA (F_{ST} = 0.125), SM and CA (F_{ST} = 0.087), ES and SM (F_{ST} = 0.073), ES and TO (F_{ST} = 0.094), SM and TO (F_{ST} = 0.060), CA and TO (F_{ST} = 0.052), CA and CO (F_{ST} = 0.124), and TO and CO (F_{ST} = 0.074) test populations.

Isozyme comparisons were conducted between pyrethroid susceptible (ST, TO, and CO) and resistant (ES) An. albimanus populations. Of the 24 enzyme systems used in the comparisons, one locus of esterase (Est-3) (Figures 3 & 4) and two loci of leucine-amino-peptidase (Lap-1 and Lap-2) (Figure 5), were found to have the same banding pattern with similarity in relative mobility between pyrethroid susceptible and resistant populations; but there was a dramatic increase in the staining intensity of esterase and leucine-amino-peptidase in the pyrethroid resistant population (ES) as compared to the susceptible populations. An increase in esterase was also detected in the SM test population.

DISCUSSION

Three important results were demonstrated from our studies. The first finding was that the old colony (Santa Tecla) showed extremely low genetic variability as compared to the five other An. albimanus populations. The second finding related to the high genetic distance occurring among the An. albimanus populations which are conspecific. The third finding concerned the pyrethroid resistance with two enzymes, esterase and leucine-amino-peptidase, that could be used to distinguish between pyrethroid susceptible and resistant populations.

Results showed that An. albimanus from the ST laboratory colony lacks polymorphic alleles for many isozymes (Table 2) compared to the other populations. Significant loss of polymorphic alleles is a result of prolonged genetic isolation of this colony. Consequently, this colony exhibited much lower levels of genetic variability than recently colonized populations (ES and SM colonies) and wild-caught populations (CA, TO, and CO populations) (Table 3). The ST colony had a low allelic polymorphism of 14.3%, while the other populations showed values ranging from 28.6 to 48.6%. One explanation is that the ST colony has been maintained in the laboratory for at least 20 years, resulting in intensive inbreeding and the disappearance of some genetic heterozygosity (Hilburn et al., 1984). The reason for the apparent loss of heterozygosity may also be an effect of the sample size used to initiate the colony. A small number of individuals used to start colony can comprise a genetic bottleneck for the colony (Munstermann, 1994).

The level of genetic variability in An. albimanus from Mexico, Guatemala, and Belize (Table 3) was similar to most other American mosquitoes (Hilburn et al., 1984), including An. albimanus from Colombia in which 25 loci and an observed heterozygosity of 0.124 were detected (Narang et al., 1991). Lower heterozygosity in the comparatively young laboratory colonies (ES and SM), as compared to wild-caught populations from Belize (CA, CO, and TO), may be due to geographical differences or to loss of

heterozygosity in colonized populations. No major allele frequency differences or fixed alleles were found among the six populations of An. albimanus. Previous studies of ribosomal DNA analysis (Beach et al., 1989b), cytogenetic analysis (Narang et al., 1991), and isozyme frequency studies (Narang et al., 1991) of An. albimanus indicated the absence of cryptic species within this species. In our studies, when two colonized populations (ES and SM) and three field populations (CA, CO and TO) were considered together, the moderate mean of F_{IS}, with the value of 0.077, indicates that random mating among the five populations of An. albimanus is occurring. The F_{ST} showed moderate differentiation among the six populations when all 35 loci were considered together. This differentiation was due mainly to the lack of heterozygosity of the ST colony (Table 4). Genetic distances among the six populations of An. albimanus were calculated, ranging from 0.019 to 0.11 (Table 5). These genetic distance values are significantly lower than the value of 0.16, which is the lower limit for conspecific population (Avise, 1975). These findings are not sufficiently strong to indicate the presence of a species complex or the speciation process of An. albimanus. We conclude that these six populations of An. albimanus show some intraspecific variations, but all populations belong to the same species.

As illustrated by the phenogram (Figure 2), the ST colony was markedly different from the other populations. In contrast, small to moderate differentiation were found among SM, ES, CA, CO, and TO. Specifically, the ES is more closely related to the SM than it is to the three populations from Belize. This may be due to the geographical proximity of populations from Guatemala and Mexico (Figure 1) resulting in greater gene flow among the populations along the Pacific Coast. Surprisingly, the CA and TO populations are more similar to the SM and ES colonies than they are to the CO population. There is no clear explanation for these relationships. However, we know that the larval habitats of An. albimanus in the Belize (Corozal) are different from the common larval habitats for other Belize (Cayo and Toledo) populations, or the Pacific Coast populations

(Rejmankova et al., 1991 & Roberts, unpublished data). These differences in larval ecology may translate into intraspecific differences. The great differentiation observed in the ST colony (Table 6) may be due to the extremely low genetic heterozygosity of the ST colony. Many isozymes detected in the ST colony were represented by a single allele.

Pyrethroids are insecticidal esters derived from primary alcohols and are thus susceptible to hydrolysis by esterases (White et al., 1976; Kerkut & Gilbert, 1985). Esterases are widely distributed in many insect tissues; such as gut, cuticle, and fat body (Kerkut & Gilbert, 1985). Elevated esterase activity has been linked to pyrethroid resistance patterns in a variety of insects. A pyrethroid-resistant southern army worm (Spodoptera eridania) showed elevated esterase activity (Abdel Aal & Soderlund, 1980). Riskallah (1983) reported that the pyrethroid-resistant Egyptian cotton leafworm (Spodoptera littoralis) showed a marked increase in esterase activity. Anopheles albimanus, resistant to deltamethrin, demonstrated elevated esterase levels in three localities from Guatemala (Beach et al., 1989a).

In our study, isozyme expression was indicated by strong staining intensities in the esterase and leucine-amino-peptidase patterns in the pyrethroid resistant population compared to the susceptible populations. Of 35 loci examined, one locus of esterase (Est-3) and two loci of leucine-amino-peptidase (Lap-1 and Lap-2) were useful for separating susceptible strains from pyrethroid resistant strain. PAGE displayed almost similar mobility as horizontal starch gel electrophoresis, confirming the similarities in mobility of Est-3 for susceptible and resistant populations. No information has been reported on leucine-amino-peptidase on insecticide resistance in mosquitoes. This finding was the first report showing that leucine-amino-peptidase is involved in pyrethroid resistance. We conclude that esterase and leucine-amino-peptidase may play an important role in pyrethroid resistance in An. albimanus vector populations. The significance of elevated esterase in a recently colonized population from Mexico (SM) is not clear because the information on its pyrethroid susceptibility was unavailable. However, we know that on the Pacific Coast of

Mexico, where this population was collected, An. albimanus is resistant to most of the insecticides that have been used both in public health and agriculture (Brogdon et al., 1988). Therefore, we surmise that the elevated esterase in SM colony may be related to earlier exposure to insecticides along the Pacific Coast. Additional work on the esterase and leucine-amino-peptidase metabolic pathways for pyrethroid resistance is required to elucidate mechanisms of pyrethroid resistance in An. albimanus.

Elevated esterase in resistant mosquitoes is also associated with various organophosphorus and carbamate compounds in the number of mosquitoes resistant to organophosphate and carbamate insecticides (Georghiou, 1986; Raymond et al., 1991). A few anophelines that are resistant to organophosphate and carbamate insecticides have shown elevated esterase. High esterase activity was found to be associated with organophosphate resistance in An. subpictus Grassi in Sri Lanka. Elevated esterases were reportedly responsible for organophosphate and pyrethroid resistance in An. albimanus from the Coastal areas of Guatemala (Beach et al., 1989a) where organophosphate and carbamate insecticides have been used for agriculture. Our findings indicated that elevated esterase was extremely high in the pyrethroid resistant Guatemalan (El Semillero) colony. Perhaps continued exposure of the An. albimanus El Semillero colony to organophosphates and carbamates may have resulted in elevated esterase levels in this pyrethroid resistant population.

Since organophosphate and carbamate susceptibility tests were not performed on the pyrethroid resistant colony (El Semillero), we do not know whether the elevated esterase level is associated only with exposure to pyrethroids, or organophosphates, or carbamates, or to all three compounds. However, based on our study, it appears that the occurrence of both enzymes in An. albimanus may limit pyrethroid use against An. albimanus populations in Central America.

REFERENCES CITED

Abdel-Aal, Y.A.I. & Soderlund, D.M. 1980. Pyrethroid-hydrolysing esterase in southern armyworm larvae: tissue distribution, kinetic properties, and selective inhibition. Pest. Biochem. Physiol. 14: 282-289.

Avise, J.C. 1975. Systematic value of electrophoretic data. Syst. Zool. 23: 465-481.

Beach, R.F., Cordon-Rosales, C. & Brogdon, W.G. 1989a. Detoxifying esterases may limit the use of pyrethroids for malaria control in the Americas. Parasitol. Today 5: 326-327.

Beach, R.F., Mills, D. & Collins, F.H. 1989b. Structure of ribosomal DNA in Anopheles albimanus (Diptera: Culicidae). Ann. Entomol. Soc. Am. 82: 641-648.

Brogdon, W.G., Beach, R.F., Stewart, J.M. & Castanaza, L. 1988. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan Anopheles albimanus. Bull. WHO 66: 339-346.

Bruce-Chwatt, L.J. 1985. Essential Malariology. Second edition. Alden Press Oxford. 452 pp.

Cavalli-Sforza, L.L. & Edwards, A.W.F. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21: 550-570

Darsie, R.F. & Ward, R.A. 1981. Identification and geographical distribution of the mosquitoes of north America, North of Mexico. Mosq. Syst. Suppl. 1: 1-313.

Ford, H.R. & Green, E. 1972. Laboratory rearing of Anopheles albimanus. Mosq. News 32: 509-513.

Georghiou, G.P. & Pasteur, N. 1978. Electrophoretic esterase patterns in insecticide-resistant and susceptible mosquitoes. J. Econ. Entomol. 71: 201-205.

Georghiou, G.P. 1986. Strategies and tactics for (resistance) management. In Pesticide Resistance. National Academy Press.

Green, C.A., Gass, R.F., Munstermann, L.E. & Baimai, V. 1990. Population genetic evidence for two species in Anopheles minimus in Thailand. Med. Vet. Entomol. 4: 25-34.

Hammock, B.D., Mumby, S.M. & Lee, P.W. 1977. Mechanism of resistance to the Juvenoid methoprene in the house fly, Musca domestica. Pestic. Bio. Chem. Physiol. 7: 261-272.

Harris, H. & Hopkinson, D.A. 1976. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland Publ. Comp., Amsterdam.

Hilburn, L.R., Willis, N.L. & Seawright, J.A. 1984. An electrophoretic comparison of laboratory colonies and natural population of Toxorhynchites (Diptera: Culicidae). J. Med. Entomol. 21: 336-343.

Kerkut, G.A. & Gilbert, L.I. 1985. Comparative Insect Physiology Biochemistry and Pharmacology. V.12 Insect control. Pergamon Press. 849 pp.

Manguin, S. & Hung, A.C.F. 1991. Developmental genetics in larvae, pupae and adults of Sepedon fuscipennis fuscipennis (Diptera: Sciomyzidae). Entomophaga 36: 183-192.

Manguin, S., Roberts, D.R., Peyton, E.L., Fernandez-Salas, I., Barreto, M., Fernandez-Loayza, R., Spinola, R.E, Granaou, R.M. & Rodriguez, M.H. 1995. Biochemical systematics and population genetic structure of Anopheles pseudopunctipennis, vector of malaria in Central and South America. Am. J. Trop. Med. Hyg. In Press.

Munstermann, L.E., 1994. Unexpected genetic consequences of colonization and inbreeding: allozyme tracking in Culicidae (Diptera). Ann. Entomol. Soc. Am. 87: 157-164.

Narang, S.K., Seawright, J.A. & Suarez, M.F. 1991. Genetic structure of natural populations of Anopheles albimanus in Columbia J. Am. Mosq. Control Assoc. 7: 437-445.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Pasteur N., Pasteur, G., Bonhomme, F. & Catalan, J. 1988. Practical Isozyme Genetics. Ellis Horwood Limited. 215 pp.

Poulik, M.D. 1957. Starch electrophoresis in a discontinuous system of buffers. Nature 180: 1477.

Raymond, M., Callaghan, A., Fort, P. & Pasteur, N. 1991. Worldwide migration of amplified insecticide genes in mosquitoes. Nature 350: 151-153.

Raymond, S. 1962. A convenient apparatus for vertical gel electrophoresis. Clin. Chem. 8: 455-470.

Rejmankova, E., Savage, M., Rejmanek, M., Arredondo-Jimenez, J.I. & Roberts, D.R. 1991. Multivariate analysis of relationships between habitats, environmental factors and occurrence of Anopheline mosquito larvae Anopheles albimanus and An. pseudopunctipennis in Southern Chiapas, Mexico. J. Appl. Ecol. 28: 827-841.

Riskallah, M.R. 1983. Esterases and resistance to synthetic pyrethroids in the Egyptian cotton leafworm. Pestic. Biochem. Physiol. 19: 184-189.

Roberts, D.R. & Andre, R.G. 1994. Insecticide resistance issues in vectors. Am. J. Trop. Med. Hyg. 50 (Suppl): 21-34.

Shaw, C.R. & Prasad, R. 1970. Starch gel electrophoresis of enzyme. A compilation of recipes. Biochem. Genetics 4: 297-320.

Swofford, D.L. & Selander, R.B. 1981. BIOSYS-1. A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Champaign, IL: Illinois Natural History Survey.

White, I.N.H., Verschoyle, R.D., Moradian, M.H. & Barnes, J.M. 1976. The relationship between brain levels of cismethrin and bioresmethrin in female rats and neurotoxic effects. Pestic. Biochem. Physiol. 6: 491-500.

World Health Organization 1992. Vector resistance to pesticides. Fifteen Report of the WHO Expert Committee on vector biology and control. WHO Tech. Rept. Ser. 818, 62 pp.

Wright, S. 1978. Evolution and Genetics of Populations. Vol. 4, University of Chicago Press, Chicago.

TABLE 1

Electrophoretic enzyme systems screened using Anopheles albimanus adults.

Enzyme system	E.C. number[1]	Symbol	No. Loci[2]	Buffer[3]
1. Aconitase	4.2.1.3	Acon	-	-
2. Acid phosphatase	3.1.3.2	Acph	1	TME
3. Adenylate kinase	2.7.4.3	Ak	2	TCss
4. Aldehyde oxidase	1.2.3.1	Ao	1	LiOH
5. Alkaline phosphatase	3.1.3.1	Aph	1	TME
6. Arginine kinase	2.7.3.3	Argk	1	LiOH
7. Esterase	3.1.1.1	Est	3	TME
8. Fumarase	4.2.1.2	Fum	1	TCss
9. Glycerol dehydrogenase	1.1.1.72	Gcd	-	-
10. Glutamate dehydrogenase	1.4.1.2	Gdh	-	-
11. Glutamate oxaloacetate transaminase	2.6.1.1	Got	2	Morph
12. ß-Glucoronidase	3.2.1.31	ß-Gn	-	-
13. Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	G3pdh	1	Morph
14. ∂-Glycerophosphate dehydrogenase	1.1.1.8	aGpdh	1	Morph
15. Glucose-6-phosphate dehydrogenase	1.1.1.49	G6pdh	1	TME
16. Glutathion reductase	1.6.4.2	Gr	2	TCss
17. ß-Hydroxyacid dehydrogenase	1.1.1.30	Had	1	Morph
18. Hexokinase	2.7.1.1	Hk	2	Morph
19. Isocitrate dehydrogenase	1.1.1.42	Idh	2	Morph
20. Lactate dehydrogenase	1.1.1.27	Ldh	1	LiOH
21. Leucine-amino-peptidase	3.4.11.1	Lap	2	LiOH
22. Malate dehydrogenase	1.1.1.37	Mdh	2	Morph
23. Malic enzyme	1.1.1.40	Me	1	TCss
24. Mannose-6-phosphate isomerase	5.3.1.8	Mpi	1	TME
25. 6-Phosphogluconate dehydrogenase	1.1.1.44	6Pgd	1	TME
26. Phosphoglucomutase	5.4.2.2	Pgm	1	Morph
27. Phosphoglucose isomerase	5.3.1.9	Pgi	2	TME
28. Pyruvate kinase	2.7.1.40	Pk	-	-
29. Sorbitol dehydrogenase	1.1.1.14	Sdh	-	-
30. Triose phosphate isomerase	5.3.1.1	Tpi	2	Morph
31. Xanthine dehydrogenase	1.2.1.37	Xdh	-	-

[1] Enzyme commission number.
[2] Number of scorable bands per phenotype.
[3] Refers to electrophoresis buffer (See materials and methods)

TABLE 2

Allele frequencies at 35 loci in six populations of Anopheles albimanus.

Locus*	ST	ES	SM	CA	TO	CO
Acph-1						
(N)	268	160	57	77	55	37
120	0.000	0.000	0.000	0.097	0.109	0.027
100	1.000	1.000	1.000	0.903	0.891	0.973
(H)	0.000	0.000	0.000	0.169	0.182	0.054
Ak-1						
(N)	156	152	57	77	55	37
100	0.353	0.704	0.675	0.442	0.691	0.473
83	0.647	0.296	0.325	0.558	0.309	0.527
(H)	0.410	0.408	0.298	0.416	0.473	0.568
Ak-2						
(N)	156	152	57	77	55	37
100	0.673	0.961	0.877	0.468	0.609	0.514
57	0.327	0.039	0.123	0.532	0.391	0.486
(H)	0.449	0.079	0.175	0.416	0.491	0.541
Ao-1						
(N)	172	160	57	77**	55	37
100	1.000	0.813	1.000	0.968	0.982	0.973
97	0.000	0.188	0.000	0.032	0.018	0.027
(H)	0.000	0.262	0.000	0.039	0.036	0.054
Aph-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Argk-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Est-1						
(N)	268	108	57	77	55	37
115	1.000	1.000	1.000	1.000	0.955	0.149
100	0.000	0.000	0.000	0.000	0.045	0.851
(H)	0.000	0.000	0.000	0.000	0.091	0.243

TABLE 2 (Continued)

Locus*	ST	ES	SM	CA	TO	CO
Est-2						
(N)	268	108	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Est-3						
(N)	268	149	57	75	55	37
100	1.000	0.554	0.658	0.113	0.318	0.270
92	0.000	0.245	0.281	0.500	0.336	0.324
85	0.000	0.201	0.061	0.307	0.264	0.162
75	0.000	0.000	0.000	0.080	0.082	0.243
(H)	0.000	0.537	0.351	0.573	0.709	0.568
Fum-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Got-1						
(N)	268	172**	57**	77**	55	37
131	0.000	0.000	0.000	0.000	0.027	0.000
110	0.000	0.070	0.009	0.000	0.064	0.000
107	0.000	0.145	0.123	0.117	0.055	0.000
100	1.000	0.323	0.816	0.818	0.673	0.770
86	0.000	0.462	0.053	0.065	0.182	0.230
(H)	0.000	0.599	0.158	0.156	0.455	0.405
Got-2						
(N)	268	172	57	77	55	37
-133	1.000	1.000	1.000	0.987	0.982	0.743
-100	0.000	0.000	0.000	0.013	0.018	0.257
(H)	0.000	0.000	0.000	0.026	0.036	0.243
Gpd-1						
(N)	202	72	57	77	55	37
100	1.000	0.979	0.982	0.974	0.936	0.892
70	0.000	0.021	0.018	0.026	0.064	0.108
(H)	0.000	0.042	0.035	0.052	0.091	0.162
G3pdh-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 2 (Continued)

Locus*	ST	ES	SM	CA	TO	CO
G6pdh-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Gr-1						
(N)	144	172	57	73	45	37
100	0.715	0.831	0.605	0.610	0.567	0.446
91	0.285	0.169	0.395	0.390	0.433	0.554
(H)	0.389	0.267	0.404	0.370	0.556	0.514
Gr-2						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Had-1						
(N)	268	172	57	77	55	37
117	0.610	0.000	0.000	0.000	0.115	0.000
100	0.390	0.198	0.658	0.455	0.555	0.649
85	0.000	0.802	0.325	0.506	0.291	0.351
50	0.000	0.000	0.009	0.032	0.000	0.000
18	0.000	0.000	0.009	0.006	0.000	0.000
(H)	0.459	0.291	0.474	0.429	0.564	0.486
Hk-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Hk-2						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Idh-1						
(N)	142	149	57	77	55**	37
118	0.042	0.081	0.219	0.149	0.291	0.189
100	0.958	0.919	0.781	0.630	0.600	0.311
92	0.000	0.000	0.000	0.221	0.109	0.500
(H)	0.070	0.148	0.404	0.532	0.582	0.622
Idh-2						
(N)	142	149	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 2 (Continued)

Locus*	ST	ES	SM	CA	TO	CO
Lap-1						
(N)	88	60	57	77	55	37
100	1.000	0.975	1.000	1.000	0.982	0.986
89	0.000	0.025	0.000	0.000	0.018	0.014
(H)	0.000	0.050	0.000	0.000	0.036	0.027
Lap-2						
(N)	88	60	57	77	55	37
120	1.000	0.600	0.674	0.325	0.721	0.824
100	0.000	0.400	0.326	0.675	0.279	0.176
(H)	0.000	0.467	0.403	0.390	0.481	0.297
Ldh-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-1						
(N)	172	99	52	69	50	37
110	1.000	0.182	0.000	0.094	0.530	0.149
100	0.000	0.818	0.923	0.841	0.470	0.500
90	0.000	0.000	0.077	0.065	0.000	0.351
(H)	0.000	0.263	0.115	0.246	0.380	0.622
Mdh-2						
(N)	172	99	99	68	50	37
-110	1.000	1.000	1.000	1.000	1.000	0.946
-100	0.000	0.000	0.000	0.000	0.000	0.054
(H)	0.000	0.000	0.000	0.000	0.000	0.108
Me-1						
(N)	268	172	57**	77**	55**	37
107	1.000	0.015	0.167	0.084	0.227	0.203
100	0.000	0.985	0.807	0.344	0.491	0.459
93	0.000	0.000	0.026	0.571	0.282	0.338
(H)	0.000	0.029	0.158	0.221	0.327	0.541
Mpi-1						
(N)	268	172	57	77	45	37
110	0.000	0.000	0.000	0.000	0.000	0.054
100	1.000	1.000	1.000	1.000	1.000	0.946
(H)	0.000	0.000	0.000	0.000	0.000	0.108

TABLE 2 (Continued)

Locus*	ST	ES	SM	CA	TO	CO
6Pgd-1						
(N)	268	172	57	77	55	37
116	1.000	1.000	1.000	0.961	0.173	0.243
100	0.000	0.000	0.000	0.039	0.636	0.568
84	0.000	0.000	0.000	0.000	0.191	0.189
(H)	0.000	0.000	0.000	0.078	0.455	0.622
Pgi-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Pgi-2						
(N)	268	172	57	77	55	37
100	1.000	1.000	1.000	1.000	1.000	1.000
(H)	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-1						
(N)	173	172	41	55	55	37**
118	0.000	0.000	0.000	0.073	0.073	0.000
100	0.864	0.866	0.854	0.782	0.782	0.743
84	0.136	0.134	0.146	0.145	0.145	0.257
(H)	0.225	0.233	0.244	0.364	0.364	0.081
Tpi-1						
(N)	268	172	57	77	55	37
100	1.000	1.000	0.982	0.974	0.982	0.986
85	0.000	0.000	0.018	0.026	0.018	0.014
(H)	0.000	0.000	0.035	0.052	0.036	0.027
Tpi-2						
(N)	268	172	57	77	55	37
120	1.000	1.000	1.000	1.000	1.000	0.973
100	0.000	0.000	0.000	0.000	0.000	0.027
(H)	0.000	0.000	0.000	0.000	0.000	0.054

* Negative values indicate cathodally migrating alleles.
** Locus deviating from Hardy-Weinberg equilibrium.
(N) Number of specimens.
(H) Heterozygosity (direct-count) per locus.
ST: Santa Tecla, ES: El Semillero, SM: Southern Mexico: CA: Cayo, TO: Toledo, CO: Corozal.

TABLE 3

Measures of genetic variability at 35 loci of six populations of Anopheles albimanus. (Standard errors are shown in parentheses).

Populations*	Mean sample size/locus	Mean no of alleles/locus	%Polymorphic loci[1]	Mean Heterozygosity direct-count	Mean Heterozygosity Hdy Wbg[2] expected[3]
1. ST	226.2	1.2	14.3	.057	.060
	(10.0)	(.1)		(.024)	(.025)
2. ES	151.1	1.5	28.6	.105	.112
	(5.9)	(.1)		(.029)	(.032)
3. SM	58.0	1.5	31.4	.093	.114
	(1.4)	(.1)		(.026)	.030)
4. CA	75.5	1.7	34.3	.129	.160
	(.7)	(.2)		(.031)	(.038)
5. TO	54.1	1.8	40.0	.181	.193
	(.4)	(.2)		(.040)	(.042)
6. CO	37.0	1.8	48.6	.200	.216
	(.0)	(.1)		(.040)	(.042)

*ST: Santa Tecla
ES: El Semillero
SM: South Mexico
CA: Cayo
TO: Toledo
CO: Corozal

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
2 HdyWbg: Hardy-Weinberg Equilibrium.
3 Unbiased estimate.

TABLE 4

F-statistic analysis of polymorphic loci in six populations of Anopheles albimanus.

Locus	F_{IS}	F_{IT}	F_{ST}
Acph-1	.043	.098	.058
Ak-1	.058	.132	.078
Ak-2	.025	.172	.078
Ao-1	.140	.227	.101
Est-1	.017	.781	.777
Fum-1	-.028	-.005	.023
Got-1	.174	.311	.166
Got-2	.310	.443	.192
Gpdh-1	.128	.158	.035
Gr-1	.049	.108	.062
Had-1	.045	.257	.221
Idh-1	-.041	.155	.188
Lap-1	-.020	-.010	.011
Lap-2	.006	.205	.200
Mdh-1	.108	.495	.434
Mdh-2	-.057	-.009	.045
Me-1	.409	.654	.414
Mpi-1	-.057	-.009	.045
6Pgd-1	.027	.543	.530
Pgm-1	.171	.186	.017
Tpi-1	-.020	-.013	.007
Tpi-2	-.028	-.005	.023
Mean	.097	.316	.243

TABLE 5

Matrix of Nei's (1978) unbiased genetic identities (above diagonal) and distances (below diagonal) in six populations of Anopheles albimanus.

Population/colony	1	2	3	4	5	6
1. ST	*****	.904	.929	.908	.934	.889
2. ES	.101	*****	.981	.957	.950	.907
3. SM	.074	.019	*****	.971	.964	.931
4. CA	.097	.044	.029	*****	.964	.938
5. TO	.069	.051	.036	.037	*****	.963
6. CO	.117	.097	.071	.064	.038	*****

*ST: Santa Tecla
ES: El Semillero
SM:South Mexico
CA: Cayo
TO: Toledo
CO: Corozal

TABLE 6

Summary of F-statistics at all loci between any of six populations of *Anopheles albimanus*.

Populations (colonies) compared*	F_{ST}**
ST vs ES	0.339
ST vs SM	0.275
ST vs CA	0.277
ST vs TO	0.161
ST vs CO	0.269
ES vs SM	0.073
ES vs CA	0.125
ES vs TO	0.094
ES vs CO	0.199
SM vs CA	0.087
SM vs TO	0.060
SM vs CO	0.157
CA vs TO	0.052
CA vs CO	0.124
TO vs CO	0.074

*ST: Santa Tecla
ES: El Semillero
SM:South Mexico
CA: Cayo
TO: Toledo
CO: Corozal

**$F_{ST} > 0.25$ Great differentiation.
$0.25 > F_{ST} > 0.15$ Moderately great differentiation.
$0.15 > F_{ST} > 0.05$ Small differentiation.
$F_{ST} \leq 0.05$ Negligible differentiation.

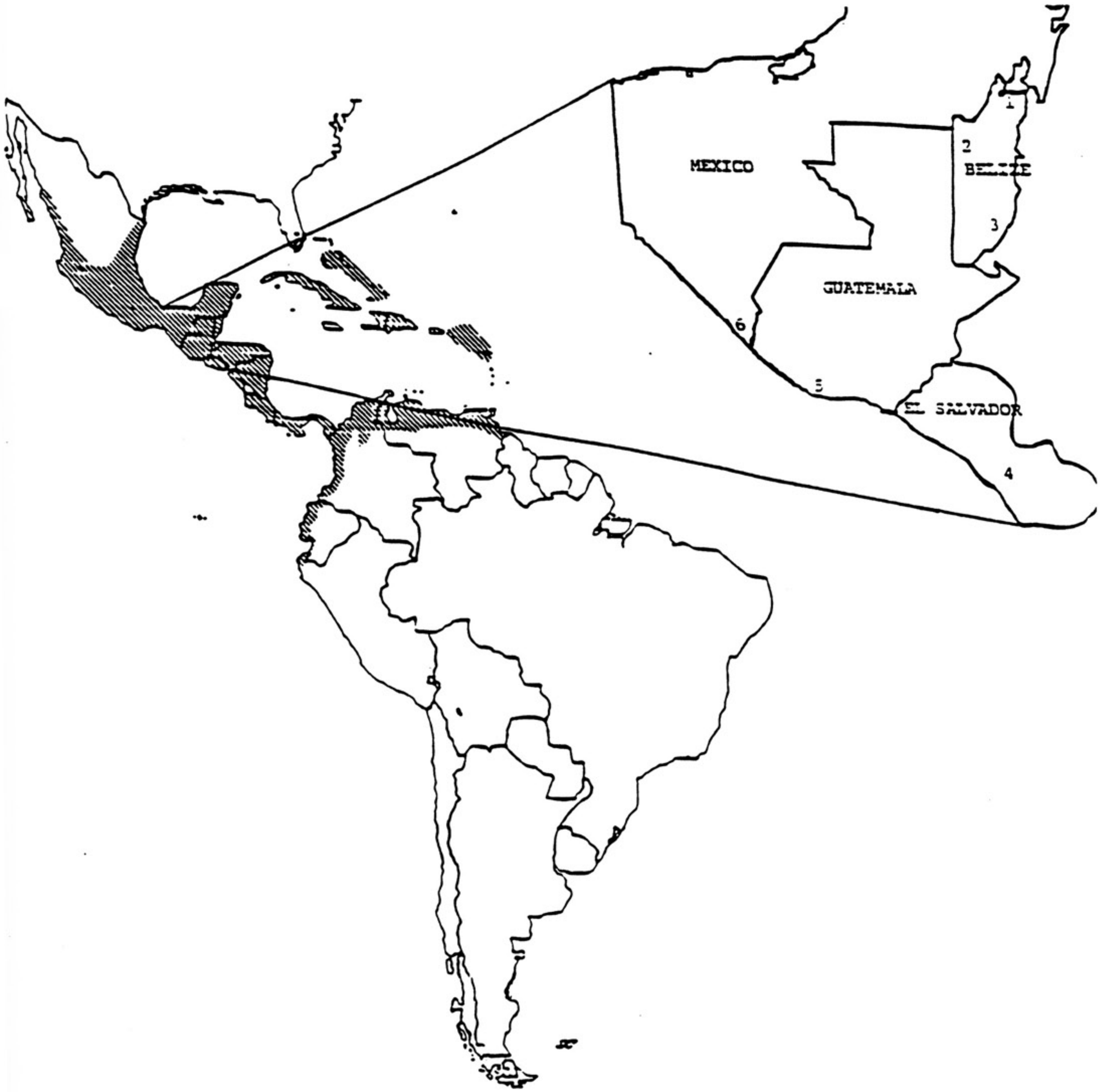


Figure 1. Collection sites of Anopheles albimanus; 1=CO (Corozal), 2=CA (Cayo), 3=TO (Toledo), 4=ST (Santa Tecla), 5=ES (El Semillero) and 6=SM (South Mexico). Hatched lines delineate the overall distribution of An. albimanus

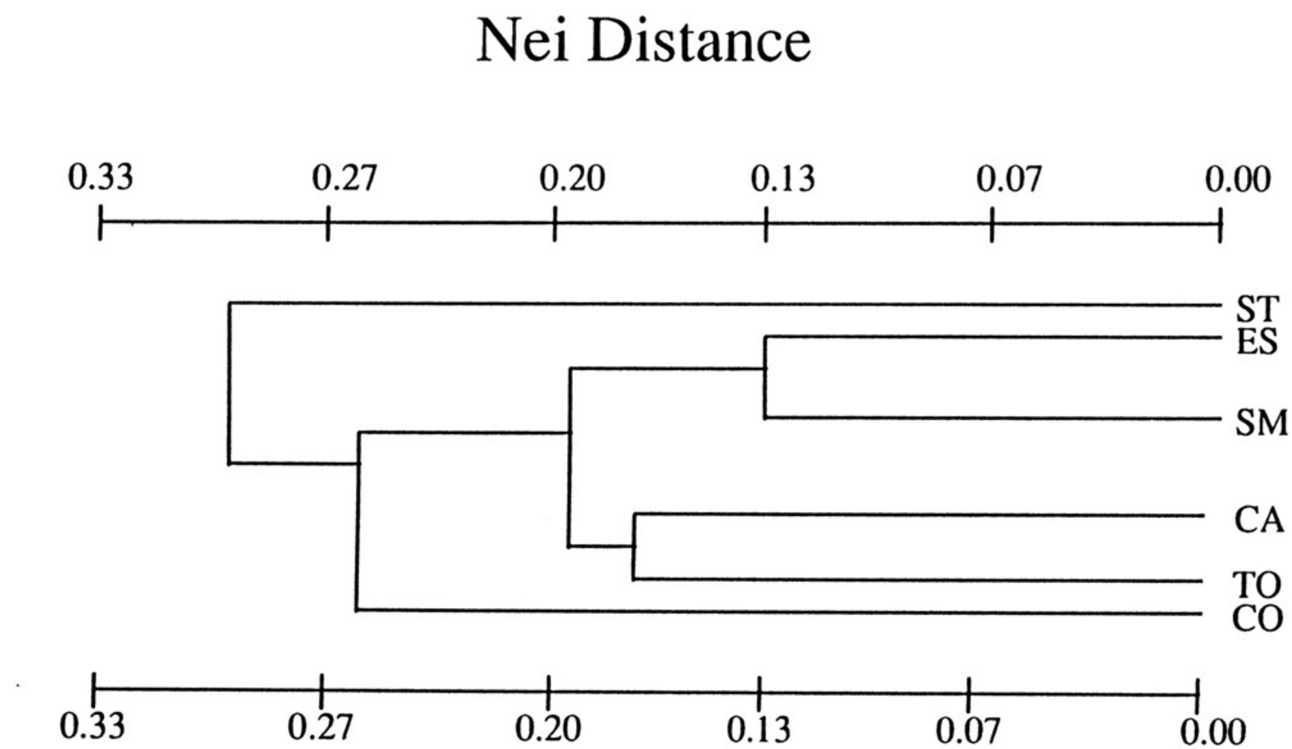


Figure 2. UPGMA phenogram from Nei's (1978) unbiased genetic distance matrix for all six populations of An. albimanus (Cophenetic correlation = 0.872). ST = Santa Tecla, ES = El Semillero, SM = South Mexico, CA = Cayo and CO = Corozal.

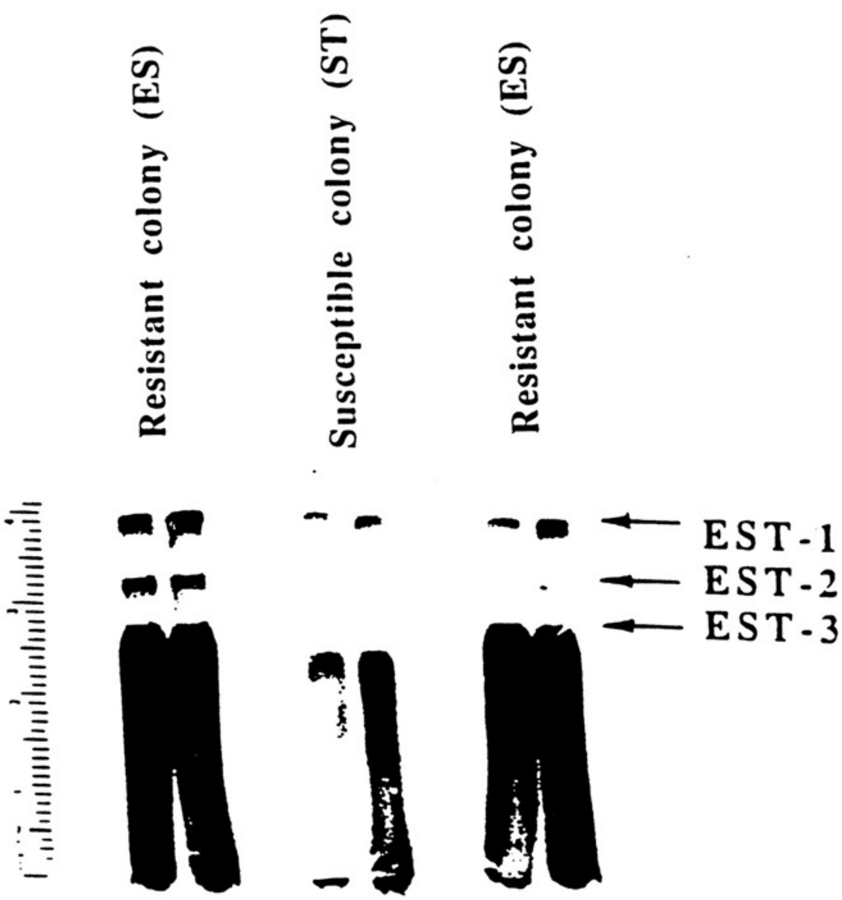


Figure 3. PAGE pattern of Est between pyrethroid resistant and susceptible populations. ST=Santa Tecla, ES=El Semillero.

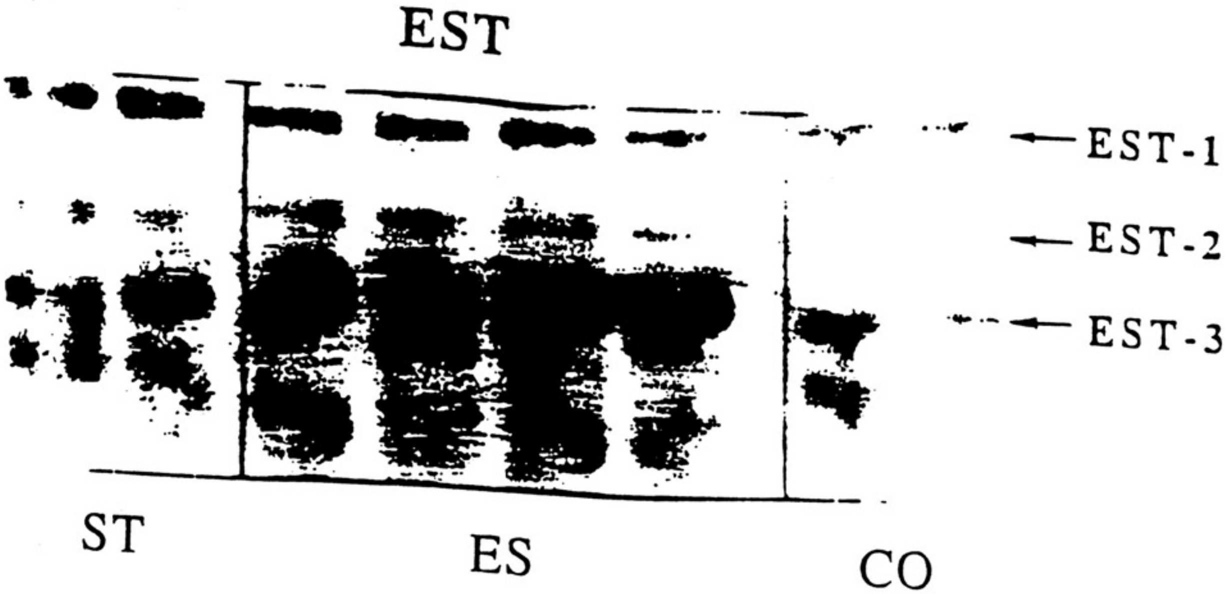


Figure 4. Starch gel electrophoresis pattern of Est between pyrethroid resistant (ES=El Semillero) and susceptible (ST=Santa Tecla and CO=Corozal) populations.

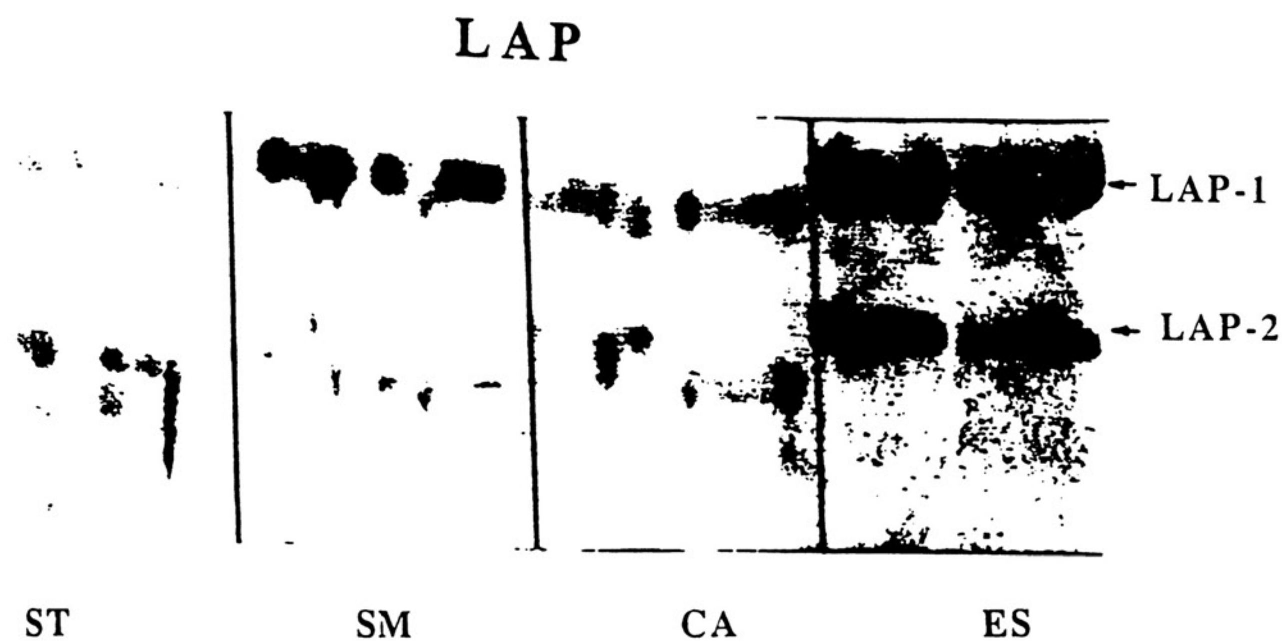


Figure 5. Starch gel electrophoresis pattern of Lap in four populations of *Anopheles albimanus* (ST=Santa Tecla, ES=El Semillero, SM=South Mexico, CA=Cayo).

CHAPTER 5

SUMMARY DISCUSSION AND CONCLUSION

SUMMARY DISCUSSION AND CONCLUSION

Two laboratory colonies of An. albimanus from El Salvador (Santa Tecla) and Guatemala (El Semillero), and two wild-caught populations from Toledo and Corozal Districts of Belize were used in our study (Table 1). The Santa Tecla colony has been maintained in the laboratory for 20 years and is physiologically susceptible to DDT, permethrin, and deltamethrin. The recently colonized population (El Semillero) from Guatemala demonstrated physiological resistance to DDT, permethrin, and deltamethrin. The field-caught specimens from Corozal were susceptible to target doses of DDT and pyrethroids; whereas, field-caught specimens from Toledo District were resistant to DDT. Multiple-resistance in the El Semillero colony (DDT, permethrin, and deltamethrin) and DDT resistance in the Toledo population were detected using the WHO recommended diagnostic dose (1975).

Toxicity of deltamethrin to larvae and adults from the Santa Tecla and El Semillero colonies is higher than that of permethrin. Comparisons of DDT, permethrin, and deltamethrin toxicities for larvae of the Santa Tecla and El Semillero colonies indicated that DDT is the most toxic compound, followed by deltamethrin and permethrin. With adults, deltamethrin shows the highest toxicity for An. albimanus, followed by permethrin and DDT.

TABLE 1. Presence of physiological resistance and behavioral avoidance in An. albimanus.

	Physiological resistance			Behavioral avoidance		
	DDT	Permethrin	Deltamethrin	DDT	Permethrin	Deltamethrin
Santa Tecla	-	-	-	-	-	-
El Semillero	+	+	+	+	+	+
Toledo	+	-	-	+	+	+
Corozal	-	-	-	+	+	+

-; absence +; presence

Isozyme analyses indicated that there were no real genetic differences among three laboratory colonies (Santa Tecla, El Semillero, and southern Mexico) and three field populations from Toledo, Cayo, and Corozal Districts in Belize, suggesting an absence of cryptic species. Our evidence that the geographic populations of An. albimanus represent a single species is in agreement with other studies that used ribosomal DNA (rDNA) analysis (Beach et al., 1989a), cytogenetic analysis (Narang et al., 1991), isozyme analysis (Narang et al., 1991), hybridization crosses (Narang et al., 1991), egg morphology (Rodriguez et al., 1992), and genetically acquired host preferences (Arredondo-Jimenez et al., 1992). Among 31 enzyme systems tested, 24 showed consistent activity and 35 loci were scorable. Higher genetic variability was found in young colonies (e.g., El Semillero) from Guatemala and Mexico and three field populations from Belize compared to the old colony from El Salvador. Of six populations examined, the old (i.e., Santa Tecla) colony showed a drastic reduction in genetic variability by displaying low allelic polymorphism. The three field populations from Belize had higher genetic variability than the laboratory colonies from Guatemala and Mexico. The Santa Tecla colony was the more extreme case,

being physiologically susceptible to all three insecticides and exhibiting no escape behavior to avoid the insecticides. In another study, Robert et al. (1991) reported that Santa Tecla females had a very low repellency response to N, N-diethyl-3-methyl-benzamide (deet) compared to females of other species. Brown (1958) found that a 20-year old laboratory colony of An. albimanus had a two times lower excitability response to DDT than wild populations of the same species. Collectively, these observations indicate that laboratory colonies can quickly lose the behavioral capacity to respond to insecticides.

Genetic isozyme expression was compared between pyrethroid resistant and susceptible populations. Of 35 loci, one locus of esterase and two loci of leucine-amino-peptidase were diagnostic of susceptible populations versus populations displaying moderate levels of pyrethroid resistance. Polyacrylamide gel electrophoresis (PAGE) was used to confirm the relative mobility of Est-3 between susceptible and resistant populations that displayed almost similar mobility on horizontal starch gels. The increase of leucine-amino-peptidase in pyrethroid-resistant arthropods has not been reported previously. This enzyme may be involved in the metabolic functions of pyrethroid resistance in arthropods. Additional work on esterase and leucine-amino-peptidase metabolic pathways of pyrethroid resistance is needed.

Pyrethroids are insecticidal esters derived from primary alcohols and are susceptible to hydrolysis by esterase (Kerkut & Gilbert, 1985). Pyrethroid-resistant Spodoptera littoralis (Egyptian cotton leafworm) was characterized with elevated esterase activity (Riskallah, 1983). Similarly, deltamethrin-resistant An. albimanus populations demonstrated elevated esterase levels in three localities of Guatemala (Beach et al., 1989b). Our findings showed a four-to-seven-fold increase in esterase activity in a pyrethroid-resistant colony of An. albimanus compared to field populations from Belize or the susceptible Santa Tecla colony from El Salvador.

Resistance to pyrethroids seems widely distributed in An. albimanus populations of Guatemala. Malcolm (1988) reported deltamethrin resistance in An. albimanus in six

localities of Guatemala, and Beach et al. (1989b) reported resistance in three localities of Guatemala. Permethrin and deltamethrin have been used by the Guatemalan malaria control programs since 1986 (Bisset et al., 1991) and the pyrethroids may act as selecting agents. Furthermore, pyrethroid resistance occurs in DDT-resistant populations as reported for an Ae. aegypti population from Thailand (Brealey et al., 1984) and Guyana (Prasittisuk & Busvine, 1977). In addition, larval pyrethroid resistance was reproduced in a DDT-selected strain of An. stephensi from Pakistan (Omar et al., 1980). In our study, the World Health Organization diagnostic test also indicated that the pyrethroid-resistant colony from Guatemala was resistant to DDT. Synthetic pyrethroid resistance may have arisen from previous exposure to DDT.

DDT was used for malaria control in the Toledo District for several years before 1990. Gramoxone, a DDT-like herbicide has been used for the weed control in rice fields, the An. albimanus habitat in Toledo District. DDT for malaria control and Gramoxone for weed control may be responsible for the DDT resistance in An. albimanus populations from Toledo District. No resistance has been detected in the Corozal population despite DDT being used in the northern Belize for malaria control. In spite of a heavy use of DDT in the past and recent use of pyrethroids in Guatemala, only a comparatively moderate level of physiological resistance, based on estimates of percent mortality (Table 2), has developed in Guatemalan colony of An. albimanus. This raises the possibility that behavioral avoidance of insecticides in these three populations serves to ameliorate the selective pressure for resistance in An. albimanus of Guatemalan as seen in An. sacharovi in Greece (de Zulueta, 1959).

TABLE 2. Percent mortality (n=3) of adult An. albimanus at the single diagnostic dosage (World Health Organization susceptibility test, 1975).

Population or colony	DDT (4.00%)	Permethrin (0.25%)	Deltamethrin (0.025%)
Santa Tecla	100	100	100
El Semillero	45	50	50
Toledo	65	100	100
Corozal	100	100	100

Muirhead-Thomson (1960) suggested the term "protective avoidance" over "behaviouristic resistance". Protective avoidance can be defined as the natural ability of mosquitoes to avoid the irritation of insecticide without previous exposure to insecticide. Behaviouristic resistance referred to a population gradually developing the ability to avoid the insecticide over time. The term "resistance" can be defined as "the developed ability in the strain of insects to tolerate doses of insecticides which prove lethal to a normal population of the same species" (World Health Organization, 1957). Therefore, the development of resistance is dependent on genetic variability already present in a population (Oppenoorth, 1984). To avoid an ambiguous interpretation, the term "behavioral avoidance" is preferred since behavioral changes by selective pressure from insecticide exposure in nature has not been adequately documented (Muirhead-Thomson, 1960).

Mechanisms of behavioral avoidance to insecticides remain a mystery since most studies have concentrated on physiological resistance mechanisms. The investigation described in this report was designed to determine if an association exists between physiological resistance and behavioral avoidance of insecticides in Anopheles albimanus

populations. Based on the results presented in Table 1, there appears to be no association between physiological resistance and behavioral avoidance in our test populations of An. albimanus. Strong behavioral avoidance was seen in three populations of An. albimanus; some of which were physiologically resistant to DDT and/or pyrethroids, i.e., no relationship between physiological resistance and behavioral avoidance was detected. Based on percent mortality at diagnostic doses of insecticides (Table 2), the El Semillero colony showed resistance to deltamethrin, permethrin, and DDT; whereas, the Belizean field population from Toledo exhibited resistance to DDT, but no pyrethroid resistance. The Santa Tecla colony and field populations from Corozal, Belize were susceptible to all insecticides. A lower degree of physiological resistance was found in field populations from Toledo compared to susceptibilities of the El Semillero colony, while Toledo populations demonstrated much stronger behavioral avoidance than the El Semillero colony. This indicates that the tendency of mosquitoes to leave DDT treated surfaces is not based solely on the level of physiological susceptibility. The Corozal population showed susceptibility to DDT and pronounced avoidance behavior. This again indicates that avoidance behavior is independent of physiological resistance; i.e., they are not necessarily inter-related as proposed by Lockwood et al., (1984). Among 80 cases of dipterans reported to show behavioral avoidance of pesticides, only 16 cases (20%) were known also to have physiological resistance (Lockwood et al., 1984). This suggests that no fixed or universal relationship between behavioral avoidance and physiological resistance is present in the Diptera.

Adult An. albimanus females from Toledo and Corozal Districts of Belize and from El Semillero of Guatemala demonstrated strong, unambiguous escape responses when placed in contact with DDT, permethrin or deltamethrin. Contact with insecticide produced a more immediate egress response and a much higher percentage of test individuals quickly escaping test chambers compared to non-contact trials. Higher doses caused more mosquitoes to escape than lower doses. Ree & Loong (1989) found that An. maculatus

showed an increased irritability response to increasing doses of permethrin. In our 30-minute exposure periods, dose levels appeared to have no effect on rate or patterns of escape in non-contact repellency tests. Dose levels did influence escape behavior in tests conducted for four-hour exposure periods. Mosquitoes from different geographic locations also show variability in escape rates as described by Busvine (1964).

Results from four-hour exposures (long term) to DDT, permethrin, and deltamethrin suggest that non-contact repellency is an important behavioral response in An. albimanus. In general, deltamethrin showed the most repellency, followed by permethrin and DDT. Unlike our finding with 30-minute exposures (short term) in non-contact trial, greater escape activity was seen at higher versus lower doses for all three compounds in four-hour exposures.

Many synthetic pyrethroids cause mosquitoes to escape sprayed houses (Miller, 1990; Lindsay et al., 1991). Our results demonstrated that both permethrin and deltamethrin produce strong behavioral escape responses from An. albimanus females. This is presumably due to the irritation caused by the chemical when mosquitoes come in contact with treated surfaces. Based on available information, it is difficult to determine how avoidance behavior originates. Avoidance behavior might be associated with selective pressure from previous exposure to synthetic pyrethroids or closely related insecticides. However, the more probable explanation is that avoidance behavior is an innate response to related classes of natural chemicals. The responses of field populations from Corozal and Toledo Districts suggest innate behavior since both populations have no history of prior exposure to synthetic pyrethroids through agricultural or public health use.

Both repellency and irritancy to DDT, permethrin, and deltamethrin were observed in An. albimanus populations. Although, true DDT repellency was documented for An. darlingi in Brazil (Roberts & Alecrim, 1991), repellency played a secondary role in the escape responses of An. albimanus females in our study. We found that DDT, permethrin, and deltamethrin have a strong irritant action and a limited repellent action on

An. albimanus females, causing them to escape exposure chambers, and to generally survive insecticide exposure. Synthetic pyrethroids had slightly stronger irritancy and repellency effects than DDT did on An. albimanus. Both repellency and irritancy effects may provide protection against indoor man-vector contact. These findings are in agreement with Roberts & Alecrim (1991) who reported DDT demonstrates a strong repellent action. A repellent action that exerts an area affect would theoretically provide significant protection from indoor transmission of malaria. However, others propose that the irritant properties of permethrin and deltamethrin in treated huts have an unsatisfactory impact on malaria vectors leading to escape and survival of mosquitoes (Rishikesh et al., 1978). This reasoning led to the termination of DDT use in many countries in Soviet Central Asia, Asia, and South Africa due to high irritancy (Bondareva et al., 1986; Sharp et al., 1990). However, malaria transmission decreases when the man-vector contact rate is reduced, and the absolute mosquito population size may not be the most important component. Additional field work is needed to study the impact of excito-repellency of insecticides and mosquito density in relation to malaria transmission.

While no method of analysis of the excito-repellency test has been fully accepted (Roberts et al., 1984), survival analysis was developed as a method of choice for excito-repellency data in this study. This analysis is advantageous by providing probability of escape over time, comparisons of response rates and patterns with exposure time. In this analysis, escaped mosquitoes are considered "dead", while the non-escaped mosquitoes are judged as "survive". A log-rank method was used for comparing the escape response behavior, and a p-value was calculated to show the differences between survival curves. This analysis was designed to minimize the loss of valuable information that might have arisen by using graphical comparisons.

Although a portable excito-repellency test chamber provided capacity to test insecticides for behavioral responses rapidly and accurately, a standard metal collapsible, excito-repellency test chamber should be developed to avoid transportation problems for the

field use. In our study, the test chambers are inexpensive and were designed for both contact and non-contact trials.

In conclusion, behavioral responses of malaria vectors to insecticides are important components of the insecticide-malaria control equation. These responses are invariably overlooked in vector control programs. Furthermore, development of resistance in malaria vectors to DDT have never been reported in some countries despite its long term use (Roberts & Andre, 1994) suggesting behavioral avoidance as the principal mechanism of control, not toxicity. More field research is needed on the behavioral responses of vector populations from different geographical locations to various insecticides. As seen from the old (Santa Tecla) colony results, we recommend that laboratory colonies should be excluded or used cautiously when conducting insecticide susceptibility and behavioral studies.

REFERENCES CITED

Arredondo-Jimenez, J.I., Bown, D.N., Rodriguez, M.H., Villarreal, C., Loyola, E.G. & Frederickson, C.E. 1992. Tests for the existence of genetic determination or conditioning in host selection by Anopheles albimanus (Diptera: Culicidae). J. Med. Entomol. 29: 894-897.

Beach, R.F., Mills, D. & Collins, F.R. 1989a. Structure of ribosomal DNA in Anopheles albimanus (Diptera: Culicidae). Ann. Entomol. Soc. Am. 82: 641-648.

Beach, R.F., Cordon-Rosales, C. & Brogdon, W.G. 1989b. Detoxifying esterases may limit the use of pyrethroids for malaria control in the Americas. Parasitol. Today 5: 326-327.

Bisset, J.A., Rodriguez, M.M., Hemingway, J., Diaz, C., Small, G.J. & Ortiz, E. 1991. The mechanisms of organophosphate and carbamate resistance in Culex quinquefasciatus from Cuba: efficacy of pirimiphos-methyl in the presence of at least three resistance mechanisms. Med. Vet. Entomol. 5: 223-228.

Bondareva, N.I., Artem'ev, M.M. & Gracheva, G.V. 1986. Susceptibility and irritability caused by insecticides to malaria mosquitoes in USSR. Part 1. Anopheles pulcherrimus Theobald. Medit. Parazitol. Parazit. Bolezni 6: 52-55.

Brealey, C.J., Crampton, P.L., Chadwick, P.R. & Rickett, 1984. Resistance mechanisms to DDT and trans-permethrin in Aedes aegypti. Pestic. Sci. 15: 121-132.

Brown, A.W.A. 1958. Laboratory studies on the behaviouristic resistance of Anopheles albimanus in Panama. Bull. WHO 19: 1053-1061.

Busvine, J.R. 1964. The significance of DDT-irritability tests on mosquitoes with an annex on results obtained with the irritability test method recommended by the WHO Expert Committee on Insecticides. Bull. WHO 31: 645-656.

de Zulueta, J. 1959. Insecticide resistance in Anopheles sacharovi. Bull. WHO 20: 797-822.

Kerkut, G.A. & Gilbert, L.I. 1985. Comparative Insect Physiology Biochemistry and Pharmacology. V.12 Insect control. Pergamon Press. 849 pp.

Lindsay, S.W., Adiamah, J.H., Miller, J.E. & Armstrong, J.R.M. 1991. Pyrethroid-treated bednet effects on mosquitoes of the Anopheles gambiae complex in The Gambia. Med. Vet. Entomol. 5: 477-483.

Lockwood, J.A., Sparks, T.C. & Story, R.N. 1984. Evolution of insect resistance to insecticides: A reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30: 41-51.

Malcolm, C.A. 1988. Current status of pyrethroid resistance in anophelines. Parasitol. Today 4: S13-S15.

Miller, J.E. 1990. Laboratory and field studies of insecticide impregnated fibers for mosquito control. Ph.D. dissertation, University of London. 336 pp.

Muirhead-Thomson, R.C. 1960. The significance of irritability behavioristic avoidance and allied phenomena in malaria eradication. Bull. WHO 22: 721-734.

Narang, S.K., Seawright , J.A. & Suarez, M.F. 1991. Genetic structure of natural populations of Anopheles albimanus in Colombia. J. Am. Mosq. Control Assoc. 7: 437-445.

Omar, S.M., Georghiou, G.P. & Irving, N. 1980. DDT-pyrethroid resistance interrelationships in Anopheles stephensi. Mosq. News 40: 200-209.

Oppenoorth, F.J. 1984. Biochemistry of insecticide resistance. Pestic. Biochem. Physiol. 22: 187-193.

Prasittisuk, C. & Busvine, J.R. 1977. DDT resistant mosquito strains with cross-resistance to pyrethroids. Pestic. Sci. 8: 527-533.

Ree, H.I. & Loong. K.P. 1989. Irritability of Anopheles farauti, An. maculatus and Culex quinquefasciatus to permethrin. Jpn. J. Sanit. Zool. 40: 47-51.

Rishikesh, N., Clarke, J.L., Mathis, H.L., King, J.S. & Pearson, J. 1978. Evaluation of decamethrin and permethrin against Anopheles gambiae and Anopheles funestus in a village trial in Nigeria. WHO/VBC/78.689.

Riskallah, M.R. 1983. Esterases and resistance to synthetic pyrethroids in the Egyptian cotton leafworm. Pestic. Biochem. Physiol. 19: 184-189.

Robert, L., Hallam, J.A., Seeley, D.C., Roberts, L.W. & Wirtz, R.A. 1991. Comparative sensitivity of 4 Anopheles (Diptera: Culicidae) to 5 repellents. J. Med. Entomol. 28: 417-420.

Roberts, D.R. & Alecrim, W.D. 1991. Behavioral response of Anopheles darlingi to DDT-spraying house walls in Amazonia. Bull. PAHO 25: 210-217.

Roberts, D.R. & Andre, R.G. 1994. Insecticide resistance issues in vectors. Am. J. Trop. Med. Hyg. 50 (Suppl) 21-34.

Roberts, D.R., Alecrim, W.D., Tavares, A.M. & McNeill, K.M. 1984. Influence of physiological condition on the behavioral response of Anopheles darlingi to DDT. Mosq. News 44: 357-362.

Rodriguez, M.H., Bown, D.N., Arredondo-Jimenez, J.I., Villarreal, C., Loyola, E.G. & Frederickson, C.E. 1992. Gonotrophic cycle and survivorship of Anopheles albimanus (Diptera: Culicidae) in Southern Mexico. J. Med. Entomol. 29: 395-399.

Sharp, B.L., le Sueur, D. & Bekker, P. 1990. Effect of DDT on survival and blood feeding success of Anopheles arabiensis in Northern Kwazulu, Republic of South Africa. J. Am. Mosq. Control Assoc. 6: 197-202.

World Health Organization 1957. World Health Expert Committee on Insecticide. 7th Report, WHO Tech. Rept. Series, No. 125.

World Health Organization 1975. Manual on Practical Entomology in Malaria. Part II. Method and techniques. Geneva, WHO Offset Publication, No 13. 191 pp.

ANNEX

BIBLIOGRAPHY

BIBLIOGRAPHY

(Bibliography related to behavioral responses)

Aboul-Nasr, A.E. 1970. Entomological problems facing malaria eradication in Africa (Uganda). Bulletin de la Societe Entomologique d' Egypte 53: 97-107.

Abraham, A.C. 1932. The anopheline mosquitoes of Hyderabad, Deccan, and their control. Records of the Malaria Survey of India 3: 179-89.

Akiyama, J. 1973. Exophily in Anopheles gambiae species B in the Sudan. Trans. R. Soc. Trop. 67: 440.

Akiyama, J. 1974. Anopheles mosquito studies in Pakistan using outlet and inlet traps. Trans. R. Soc. Trop. 68: 14-15.

Alekseev, A.N., Drobozina, V.P., Bondareva, N.I. & Varenik, L.I. 1983. Aktivnost krovososaniia rezistentnykh k DDT populiatsii Anopheles messeae v otsutstvie i pri nalichii kontakta s DDT [Blood-sucking activity of DDT-resistant populations of Anopheles messeae in the absence and presence of contact with DDT]. Med. Parazitol. (Mosk) 5: 72-77.

Annecke, S. 1950. Economic importance of malaria. Department of Health, Malaria Research and Control Station, Tzaneen, Transvaal. WHO/Mal/Conf; 13: 1-8 pp.

Aragao, M.B. 1964. Distribuicao geografica e abundancia das especies de Anopheles (Kerteszia) (Diptera, Culicidae). Rev. Bras. Malariol. Doencas. Trop. 16: 73-109.

Aragao, M.B. 1968. O ciclo anual dos Anopheles do subgenero Kerteszia, no sul do Brasil. Memorias do Instituto Oswaldo Cruz 66: 85-106.

Aragao, M.B. 1974. O comportamento dos anofelinos do subgenero kerteszia, no sul do Brasil e o efeito do inseticida DDT [The behavior of anophelines of the subgenus kerteszia in south Brazil, and the effect of the insecticide DDT]. Memorias do Instituto Oswaldo Cruz. 72: 147-72.

Arnold, P. & Moray, N. 1964. Confirmation of apparent genetic assimilation of behavior in Drosophila melanogaster. Nature 204: 504.

Artemyev, M.M., Bondareva, N.I. & Kulkova, T.A.. 1987. Susceptibility and irritability by insecticides in anophelines of the USSR. Communication 2: Anopheles superpictus Grassi in the Tadjik SSR. Med. Parazitol. (Mosk) 1: 8-10.

Artemyev, M.M., Sorokin, N.N., Bondareva, N.I., Aliev, A.I. & Adamishina, T.A. 1989. Nasledovanie razdrazhimosti k insektitsidam u maliariinykh komarov [The inheritance of irritability to insecticides in malarial mosquitoes]. Med. Parazitol. (Mosk) 6: 39-43.

Ault, S.K. 1983. Anthropological aspects of malaria control planning in Sri Lanka. Med. Anthropology: 27-50.

Austel, V. & Kutter, E. 1980. Practical Procedures in Drug Design. In: Ariens, E.J., editor. Drug Design. New York: Academic Press, 69 pp.

Avlavidov, T. & Kovchazov, T.G. 1966. On the sensitivity of Anopheles to DDT in the district of the city of Varna. Epid. Mikrobiology Infekiozni Bolesti Sofiia. 3: 151-153.

Bakri, G. & Noguer, A. 1977. Impact of a single DDT spraying application on malaria vector densities and entomological inoculation rates in Togo. WHO/VBC.77.676: 1-10.

Bang, Y.H. 1985. Implication in the control of malaria vectors with insecticides in tropical countries of South-East Asia region: Part 2, Consequences of insecticide use. J. Commun. Dis.17: 300-310.

Bar-Zeev, M. 1965. Some effects of dichlorvos in huts on the behavior of mosquitoes in Southern Nigeria. Bull. WHO 33: 583-86.

Barai, D., Hyma, B. & Ramesh, A. 1982. The scope and limitations of insecticide spraying in rural vector control programmes in the states of Karnataka and Tamil Nadu in India. Ecol. Dis. 1: 243-55.

Barber, G.W. & Schmitt, J.B. 1949. Further studies on resistance to DDT in the housefly. J. Econ. Entomol. 42: 287-92.

Barbosa, P. & Letourneau, D.K. 1988. Novel aspects of insect-plant interactions. New York: John Wiley & Sons.

Beach, R.F., Brogdon, W.G., Castanaza, L.A., Cordon-Rosales, C. & Calderon, M. 1989. Effect of temperature on an enzyme assay to detect fenitrothion resistance in Anopheles albimanus mosquitoes. Bull. WHO 67: 203-8.

Bertram, D.M. 1950. A critical evaluation of DDT and 'gammexane' in malaria control in Upper Assam over five years, with particular reference to their effect on Anopheles minimus. Ann. Trop. Med. Parasitol. 44: 242-54.

Bhatia, S.C. & Deobhankar, R.B. 1962. Irritability of susceptible and resistant field populations of Anopheles culicifacies in Maharashtra state, India. WHO/MAL. Oct 12: 1-9.

Bhombore, S.R., Sitaraman, N.L. & Achuthan, C. 1956. Studies on the bionomics of Anopheles fluviatilis in Mysore state, India: 2. Bionomics in Western Hill-tracts, Mysore state. Indian. J. Malariol. 10: 23-32.

Bilbie, I., Enescu, A., Giurca, I., Nicolescu, G. & Velehorschi, N. 1983. Laboratory and field investigations on insecticide susceptibility of the anophelines in the previously endemic areas of malaria in the Danube Plain and Dobrudja. Archives Roumaines de Pathologie Experimentale et de Micribiologie 42: 343-351.

Birley, M.H. & Charlewood, J.D. 1987. Sporozoite rate and malaria prevalence. Parasitol. Today 3: 231-232.

Blair, D.M. 1950. Report on malaria control in southern Rhodesia. WHO/MAL. 48: 1-8.

Bonair, A., Rosenfield, P. & Tengvald, K. 1989. Medical technologies in developing countries: issues of technology development, transfer, diffusion and use. Soc. Sci. Med. 28: 769-81.

Bondareva, N.I., Drobozina, V.P. & Artemyev, M.M. 1984. Various species of Anopheles in the USSR and their irritability due to insecticides. Med. Parazitol. (Mosk) 4: 46-50.

Bordas, E. & Downs, W.G. 1951. Control of Anopheles pseudopunctipennis in Mexico with DDT residual sprays applied in buildings. Part 4: activity pattern of adult An. pseudopunctipennis Theo. Am. J. Hyg. 53: 217-223.

Bordasch, R.P. & Berryman, A.A. 1977. Host resistance to the fir engraver beetle, Scolytus ventralis (Coleoptera: Scolytidae) 2. Repellency of Abies grandis resins and some monoterpenes. Can. Entomol. 109: 95-100.

Bown, D.N., Rios, J.R., del Angel Cabanas, G., Guerrero, J.C. & Mendez, J.F. 1984. Evaluation of chlorphoxim used against Anopheles albimanus on the south coast of Mexico: 1. Results of indoor chlorphoxim applications and assessment of the methodology employed. Bull. PAHO 18: 379-88.

Bown, D.N., Rios, J.R., del Angel Cabanas, G., Guerrero, J.C. & Mendez, J.F. 1985. Evaluation of chlorphoxim used against Anopheles albimanus on the coast of Mexico: 2. Use of two curtain-trap techniques in a village-scale evaluation trial. Bull. PAHO 19: 61-68.

Bown, D.N., Rios, J.R., Frederickson, C., Del Angel Cabanas, G. & Mendez, J.F. 1986. Use of an exterior curtain-net to evaluate insecticide/mosquito behavior in houses. J. Am. Mosq. Control Assoc. 2: 99-101.

Bradley, A.K., Greenwood, A.M., Byass, P., Greenwood, B.M., Marsh, K., Tulloch, S. & Hayes, R. 1986. Bed-nets (mosquito-nets) and morbidity from malaria. Lancet 26: 204-207.

Breeland, S.G. 1974. Population patterns of Anopheles albimanus and their significance to malaria abatement. Bull. WHO 50: 307-315.

Bret, B.L. & Ross, M.H. 1985. Insecticide-induced dispersal in the German cockroach, Blattella germanica (L.) (Orthoptera: Blattellidae). J. Econ. Entomol. 78: 1293-1298.

Bret, B.L. & Ross, M.H. 1986. Behavioral responses of the German cockroach, Blattella germanica (L.) (Orthoptera: Blattellidae), to a propoxur formulation. J. Econ. Entomol. 79: 426-430.

Brown, A.W.A. 1958. Laboratory studies on the behavioristic resistance of Anopheles albimanus in Panama. Bull. WHO 19: 1053-1061.

Brown, A.W.A. 1958. Quantitative measurements towards an understanding of behavioristic resistance in Panama. B. In the laboratory. In: WHO PAHO Sem. on Suscept. Insects to Insectic. 323-331.

Brown, A.W.A., Haworth, J. & Zahar, A.R. 1976. Malaria eradication and control from a global standpoint. J. Med. Entomol. 13: 1-25.

Brown, A.W.A. 1959. Inheritance of insecticide resistance and tolerance. Misc. Publ. Entomol. Soc. Am. 1: 20-26.

Brown, A.W.A. 1983. Insecticide resistance as a factor in the integrated control of Culicidae. In Integrated Mosquito Control Methodologies. Volume 1. Laird, M. & Miles, J. Eds. Academic Press, New York. 161-235.

Browne, L.B. 1979. Host-related responses and their suppression: some behavioral considerations. In: Shorey, H. H. & McKelvey, J. J., editors. Chemical Control of Insect Behavior: Theory and Application. New York, N. Y. John Wiley & Sons: 117-127.

Bryan, J.H., Petrarca, V., Di Deco, M.A. & Coluzzi, M. 1987. Adult behavior of members of the Anopheles gambiae complex in the Gambia with special reference to An. melas and its chromosomal variants. Parassitologia. 29: 221-249.

Buescher, M.D., Rutledge, L.C. & Wirtz, R.A. 1987. Studies on the comparative effectiveness of permethrin and deet against bloodsucking arthropods. Pest. Sci. 21: 165-173.

Buescher, M.D., Rutledge, L.C., Wirtz, R.A. & Nelson, J.H. 1983. The dose-persistence relationship of deet against Aedes aegypti. Mosq. News 43: 364-366.

Bunker, C.W.O. & Hirschfelder, A.D. 1925. Mosquito repellents. Am. J. Trop. Med. Hyg. 5: 359-383.

Burden, G.S. 1975. Repellency of selected insecticides. Pest Control 43: 16-18.

Busvine, J.R. & Nash, R. 1953. The potency and persistence of some new synthetic insecticides. Bull. Entomol. Res. 44: 371-376.

Busvine, J.R. 1963. The significance of DDT irritability tests with mosquitoes, with some results obtained with the WHO provisional test method as modified by Mr.Coluzzi. WHO/VBC. 21: 1-23.

Busvine, J.R. 1964. The significance of DDT-irritability tests on mosquitoes. Bull. WHO 31: 645-656.

Buttiker, W. 1958. Notes on exophily in anophelines in South-East Asia. Bull. WHO 19: 1118-1123.

Buxton, P.A. 1952. The place of insecticides in tropical medicine: an introduction. Trans. R. Soc. Trop. 46: 216-226.

Calderon, G.R. 1988. Estado actual de los plaguicidas en El Salvador. PAHO Regional Conference. 29 pp.

Carrillo, H.F. 1988. Empleo de insecticidas en salud Publica Estados Unidos Mexicanos, 1957-1987. PAHO Regional Conference. 4 pp.

Carrillo, H.F. 1988. Empleo de plaguicidas para fines agricolas Estados Unidos Mexicanos, 1984-1988. PAHO Regional Conference. 13 pp.

Caspari, E. 1963. Genes and the study of behavior. Am. Zool. 3: 97-100.

Caspari, E. 1964. Refresher course on behavior genetics: introductory remarks. Am. Zool. 4: 97-99.

Catangui, F.P., Valera, C.V. & Cabrera, B.D. 1985. Vectors of malaria in the Philippines, southeast Asian. J. Trop. Med. Publ. Hlth. 16: 139-140.

Cefalu, M, Oddo, F. & Sacca, G. 1961. Vita extra-domestica di Anopheles labranchiae in Sicilia. Osservazioni in un'area di sospensione dei trattamenti con DDT. Parassitologia. 3: 23-51.

Chang, T.L. 1940. The anopheline mosquitoes of Yunnan: notes on their breeding habits and adult behavior. Chinese Med. J. 58: 218-233.

Charlwood, J. D. & Paraluppi, N. D. 1978. O uso de caixas excito-repelentes com Anopheles darlingi Root, An. nuneztovari Gabaldon e Culex pipiens quinquefasciatus Say, obtidos em areas perto de Manaus, Amazonas [The use of excito-repellency boxes with Anopheles darlingi Root, A. nuneztovari Gabaldon and Culex pipiens quinquefasciatus Say, obtained from areas near Manaus, Amazonas]. Acta Amazonica. 8: 605-611.

Charlwood, J.D. & Dagoro, H. 1987. Impregnated bed nets for the control of filariasis transmitted by Anopheles punctulatus in rural Papua New Guinea. PNG. Med. J. 30: 199-202.

Chinery, W.A. 1969. A survey of mosquito breeding in Accra, Ghana, during a two year period of larval mosquito control. 1. The mosquitoes collected and their breeding places. Ghana. Med. J. 266-74.

Chinery, W.A. 1970. A survey of mosquito breeding in Accra, Ghana, during a two-year period of larval mosquito control. 2. The breeding of Culex (Culex) pipiens fatigans and Anopheles (Cellia) gambiae, in Accra. Ghana Med. J. 109-116.

Choudbury, D.S. & Rahman, S.J. 1967. Observation on the irritability of susceptible and resistant strains of An. stephensi to DDT. Bull. Indian. Soc. Malar. Commun. Dis. 4: 129-134.

Chow, C.Y. 1956. DDT-resistance of Anopheles sundaicus in Java. Bull. WHO 15: 785-786.

Chow, C.Y. 1969. Ecology of malaria vectors in the Pacific. Cah. Orstrom. Ser. Entomol. Med. Parasitol. 7: 93-97.

Clyde, D.F. 1987. Recent trends in the epidemiology and control of malaria. Epid. Rev. 9: 219-243.

Colless, D.H. 1953. The effect of DDT and kerosene upon adult behavior in Anopheles balabacensis Baisas (An. leucosphyrus Auct.). Ann. Trop. Med. Parasitol. 47: 261-271.

Collins, R.T., Jung, R.K., Anoez, H., Sutrisno, R.H. & Putut, D. 1979. A study of the coastal malaria vectors, Anopheles sundaicus (Rodenwaldt) and Anopheles subpictus Grassi, in south Sulawesi, Sulawesi, Indonesia. WHO/VBC.79.740: 1-12.

Coluzzi, A. & Coluzzi, M. 1961. Sull' irritabilita degli Anopheles al DDT in rapporto all'eradicazione della malaria [The irritant effect of DDT on Anopheles with reference to the eradication of malaria]. Riv. Malariol. 40: 35-40.

Coluzzi, A., Cullen, J.R. & de Zulueta, J. 1962. A field trial in Uganda of a new method for determining the irritability of mosquitoes to insecticides (preliminary report). WHO/Insecticides 2: 1-9.

Coluzzi, M. 1962. An experimental method for determining the irritability of adult mosquitoes to insecticides. WHO/Insecticides 30: 1-15.

Coluzzi, M. 1963. Studies on irritability of DDT to anopheline mosquitoes. WHO/VBC. 25: 1-22.

Coluzzi, M. 1963. Sulla irritabilita al DDT in Anopheles [On the irritability of Anopheles to DDT]. Riv. Malariol. 42: 189-222.

Consolim, J., Luz , E., Vieira, A.M. & Borba, A.M. 1979. Alguns aspectos epidemiologicos sobre a persistencia de transmissao de malaria no litoral Paranaense. 3) Entrada a permanencia de Anopheles cruzii na habitacao humana (Diptera, Culicidae). Arq. Biol. Tecnol. 22: 173-179.

Correa, R.D.R., Ferreira, E., Ramalho, R.G. & Zaia, L. 1971. Suscetibilidade do Anopheles darlingi, vector de malaria, aos inseticidas. Rev. Bras. Malariol. Doencas. Trop. 23: 105-110.

Correa, R.R., Guarita, O.F. & Costa, F.D.A. 1964. O Anopheles cruzii e o A. bellator na campanha de erradicacao da malaria em Sao Paulo [Behavior of Anopheles cruzii and An. bellator in the malaria eradication campaign in Sao Paulo]. Arquivos de Higiene e Saude Publica. 29: 207-219.

Cova-Garcia, P. 1958. Manifestations of behavioristic resistance in Venezuela. In: WHO PAHO Sem. on Suscept. Insects to Insectic. 295-303.

Coz, J. & Hamon, J. 1963. Importance pratique de la resistance aux insecticides en Afrique au sud de Sahara pour l'eradication du paludisme dans ce continent. Cah. Orstrom. Ser. Entomol. Med. Parasitol. 1: 27-37.

Cullen, J.R. & de Zulueta, J. 1962. Observations on the irritability of mosquitoes to DDT in Uganda. Bull. WHO 27: 239-250.

Cullen, J.R. & de Zulueta, J. 1963. Observations on the effect of residual insecticides in experimental huts in Masaka district (Uganda). WHO/VBC. 38: 1-22.

Curtis, C.F. & Hill, N. 1988. Comparison of methods of repelling mosquitoes. Entomologia Experimentalis et Applicata. 49: 175-179.

Curtis, C.F. & Lines, J.D. 1985. Impregnated fabrics against malaria mosquitoes. Parasitol. Today 1: 147.

Curtis, C.F. & Lines, J.D. 1987. Insecticides in the management of insect vectors of tropical disease. Insect Sci. Appl. 8: 709-14.

Curtis, C.F. 1986. Fact and fiction in mosquito attraction and repulsion. Parasitol. Today 316-318.

Curtis, C.F., Lines, J.D., Ijumba, J., Callaghan, A., Hill, N. & Karimzad, M.A. 1987. The relative efficacy of repellents against mosquito vectors of disease. Med. Vet. Entomol. 1: 109-119.

D'Allessandro, G. 1954. Studio di un habitat montano dell' A. labranchiae in Sicilia. Rivista di Parassitologia. 15: 361-372.

Dabrowski, Z.T. & Rodriguez, J.G. 1971. Studies on resistance of strawberries to mites. 3. Preference and non preference responses of Tetranychus urticae and T. turkestani to essential oils of foliage. J. Econ. Entomol. 64: 387-391.

Daggy, R.H. 1959. Malaria in oases of eastern Saudi Arabia. Am. J. Trop. Med. Hyg. 8: 223-291.

Dahm, P.A. 1957. The mode of action of insecticides exclusive of organic phosphorus compounds. Annu. Rev. Entomol. 2: 247-60.

Davidson, G. & Jackson, C.E. 1961. Insecticide resistance in mosquitoes. Nature 190: 364-65.

Davidson, G. & Zahar, A.R. 1973. The practical implications of resistance of malaria vectors to insecticides. Bull. WHO 49: 475-483.

Davidson, G. 1953. Experiments on the effect of residual insecticides in houses against Anopheles gambiae and A. funestus. Bull. Entomol. Res. 44: 231-255.

de Bustamante, F.M. 1949. Da aplicacao intradomiciliar de DDT no combate a malaria transmitida por Kerteszia. Rev. Bras. Malariol. Doencas. Trop. 1: 205-210.

de Bustamante, F.M. 1957. Distribuicao geografica e periodicidade estacional da malaria no Brasil e sua relacao com os fatores climaticos. Situacao atual do problema. Rev. Bras. Malariol. Doencas. Trop. 9: 181-90.

de Bustamante, F.M., Pinto, O.S., Guedes, A.S., Xavier, S.H. & Freitas, J.R. 1951. Sobre a captura do Anopheles darlingi e do Anopheles albitarsis nas paredes externas de casas dedetizadas em Engenheiro Dolabela, Minas Gerais. Rev. Bras. Malariol. Doencas. Trop. 3: 122-129.

de Bustamante, F.M., Pires, W.M. & Gusmao, J.B. 1950. Sobre a densidade do A. tarsimaculatus Goeldi, 1905 (= A. aquasalis Curry, 1932) em area da Baixada Fluminense sujeita a aplicacoes intradomiciliares de DDT. Rev. Bras. Malariol. Doencas. Trop. 2: 266-271.

de Zulueta, J. & Cullen, J.R. 1963. Deterrent effect of insecticides on malaria vectors. Nature 200: 860-861.

de Zulueta, J. 1952. Observations on mosquito density in an endemic malarious area in eastern Colombia. Am. J. Trop. Med. Hyg. 1: 314-329.

de Zulueta, J. 1956. Malaria in Sarawak and Brunei. Bull. WHO 15: 651-671.

de Zulueta, J. 1959. Insecticide resistance in Anopheles sacharovi. Bull. WHO 20: 797-822.

de Zulueta, J. 1962. The irritability of mosquitoes to DDT and its importance in malaria eradication. Rivista di Malariologia 41: 169-78.

de Zulueta, J., Chang, T.L., Cullen, J.R. & Davidson, G. 1968. Recent observations on insecticide resistance in Anopheles stephensi in Iraq. Mosq. News 28: 499-503.

de Zulueta, J., Jolivet, P., Thymakis, K. & Caprari, P. 1957. Seasonal variations in susceptibility to DDT of Anopheles maculipennis in Iran. Bull. WHO 16: 475-479.

de Zulueta, J., Kafuko, G.W., Cullen, J.R. & Pedersen, C.K. 1961. The results of the first year of a malaria eradication pilot project in northern Kigezi (Uganda). East. Afr. Med. J. 38: 1-26.

Dethier, V.G., Browne, L.B. & Smith, C.N. 1960. The designation of chemicals in terms of the responses they elicit from insects. J. Econ. Entomol. 53: 134-36.

Dharmshaktu, N.S., Prabhakaran, P.K. & Menon, P.K.M. 1987. Laboratory study on the mosquito larvicidal properties of leaf and seed extract of the plant. J. Trop. Med. Hyg. 90: 79-82.

Diaz Najera, A. 1964. Nueva tecnica para ensayos de formulaciones de insecticidas sobre Anopheles [A new method for the testing of insecticides on Anopheles]. Revista del Instituto de Salubridad y Enfermedades Tropicales 24: 31-42.

Dowling, M.A.C. 1955. Insect repellents and miticides. J. R. Army Med. Corps. 101: 1-15.

Downs, W.G.& Bordas, E. 1951. Control of Anopheles pseudopunctipennis in Mexico with DDT residual sprays applied in buildings. Part 5: effectiveness of residual applications of DDT and gammexane up to one year after application under controlled conditions. Am. J. Hyg. 54: 150.

Drobozina, V.P., Alekseev, A.N. & Bondareva, N.I. 1980. Attack activity of DDT-resistant Anopheles labranchiae-atroparvus after contact with this insecticide. Med. Parazitol (Mosk) 49: 9-12.

Drobozina, V.P., Alekseev, A.N., Bondareva, N.I. & Suvorova, N.I. 1977. Razdrezhimost' i povedenie Anopheles sacharovi Favre v obrabotannykh pomeshcheniiakh poselkov Azerbaidzhanskoi SSR [Irritability and behavior of Anopheles sacharovi Favre in treated dwellings in villages of the Azerbaijan SSR]. Med. Parazitol. (Mosk) 46: 51-57.

Drobozina, V.P., Artemyev, M.M. & Kashayeva, G.V. 1984. [Sensitivity to insecticides (DDT and malathion) and the irritability of them exposed to malarial mosquitoes of natural population in the Dagestan ASSR]. Med. Parazitol. (Mosk) 44-46.

Drobozina, V.P., Bondareva, N.I., Alekseev, A.N. & Kashaeva, G.V. 1988. Povedenie komarov Anopheles pri kontakte s insektitsidami [Behavior of Anopheles mosquitoes in contact with insecticides]. Parazitologiia 22:137-142.

Duret, J.P. 1958. Quantitative measurements toward an understanding of behavioristic resistance in Panama (in the field). In: WHO PAHO Sem. on Suscept. Insects to Insectic 318-322.

Duret, J.P. 1961. Estudios sobre el comportamiento de los Anofeles del Rio Chagres, Panama. Boletin de la Oficina Sanitaria Panamericana. 51: 285-302.

Duret, J.P. 1964. Pruebas de excito-repelencia de algunas anofelinos de Panama [Excito-repellency tests for some anophelines from Panama]. Bol Oficina Sanitaria Panamerica 57: 259-267.

Ebeling, W., Reierson, D.A. & Wagner, R.E. 1967. Influence of repellency on the efficacy of blatticides. 2. Laboratory experiments with German cockroaches. J. Econ. Entomol. 60: 1375-1390.

Ebeling, W., Reierson, D.A. & Wagner, R.E. 1968. The influence of repellency on the efficacy of blatticides. 3. Field experiments with German cockroaches with notes on three other species. J. Econ. Entomol. 61: 751-61.

Ejercito, A., Hess, A.D. & Willard, A. 1954. The six-year Philippine-American malaria control program. Am. J. Trop. Med. Hyg. 3: 971-980.

el Gaddal, A.A., Haridi, A.M., Hassan, F.T. & Hussein, H. 1985. Malaria control in the Gezira-Managil irrigated scheme of the Sudan. J. Trop. Med. Hyg. 88: 153-159.

el Samani, F.Z., Willett, W.C. & Ware, J.H. 1987. Nutritional and socio-demographic risk indicators of malaria in children under five: a cross-sectional study in a Sudanese rural community. J. Trop. Med. Hyg. 90: 69-78.

el-Sawy, M.F., el-Hamd, Z.M., Loutfy, N.F., el Masry, S. & Abdel-Gualil, M.Z. 1986. Ambrosia maritima a larvicide and pupacide for Anopheles pharoensis ambrosin as an effective insecticide. 1. Laboratory experimentation. J. Egypt. Soc. Parasitol. 16: 57-64.

Elliott, R. & de Zulueta, J. 1975. Ethological resistance in malaria vectors. Behavioral response to intradomestic residual insecticides. WHO/VBC75.569: 1-15.

Elliott, R. 1964. Studies on the kinetic response of mosquitoes to chemicals. Bull. WHO 31: 657-667.

Elliott, R. 1968. Studies on man-vector contact in some malarious areas in Colombia. Bull. WHO 38: 239-53.

Elliott, R. 1969. Ecology and behavior of malaria vectors in the American region. Cah. Orstrom. Ser. Entomol. Med. Parasitol. 7: 29-33.

Elliott, R. 1972. The influence of vector behavior on malaria transmission. Am. J. Trop. Med. Hyg. 21: 755-763.

Ermishev, Y.U.V., Kul'Kova T.A. & Polevoin, I. 1977. A study of sensitivity and irritability of mosquitoes of the genus Anopheles in relation to insecticides in Northeastern Afghanistan. Med. Parazitol. (Mosk) 4: 444-47.

Esenther, G.R. & Beal, R.H. 1978. Insecticidal baits on field plot perimeters suppress Reticulitermes. J. Econ. Entomol. 71: 604-607.

Eshghy, N. & Laarman, J.J. 1977. Laboratory experiments on the irritability of Anopheles atroparvus and Anopheles stephensi to DDT. Iranian. J. Publ. Hlth. 6: 12-23.

Espinosa, J., Ferrer, A. & Montenegro, L. 1988. Diagnostico sobre uso de plaguicidas por productores nacionales. PAHO Regional Conference, 22 pp.

Ewing, A.W. 1963. Attempts to select for spontaneous activity in Drosophila melanogaster. Animal Behaviour 11: 369-378.

Fakir, M.T. 1957. Rice field malaria and its control. Bull. Endem. Dis. 2: 15-18.

Farid, M.A. 1956. Implications of the Mecca pilgrimage for a regional malaria programme. Bull. WHO 15: 828-33.

Fay, R. & Sheppard, E. 1949. Anopheles quadrimaculatus activity patterns in the laboratory on untreated and DDT-treated surfaces. J. Nat. Mal. Soc. 8: 147-58.

Fay, R. 1970. In discussion of Hamon, J., Mouchet, J., Brengues, J., & Chauvet, G. Vector ecology and behavior before, during, and after application of control measures. Misc. Publ. Entomol. Soc. Am. 7: 43.

Feng, L.C. 1938. A critical review of literature regarding the records of mosquitoes in China. Peking. Nat. Hist. Bull. 12: 294-296.

Fischer, L. & Steinhart, W. 1957. Malaria und malariaubertrager in Sarobie (Afghanistan). Zeitschrift fur Tropenmedizin und Parasitologie 8: 69-83.

Fletcher, B.S. 1979. Behavioral responses of Diptera to pheromones, allomones, and kairomones. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N.Y. John Wiley & Sons. 129-148.

Fontaine, R.E. 1978. House spraying with residual insecticides with special reference to malaria control. WHO/VBC78.704: 1-28.

Fontaine, R.E., Houng, A.Y. & Van Severin, M. 1986. Household aggregation of malaria for assessing geographic diversity of malaria for control programs. 21 pp.

Fontaine, R.E., Joshi, G.P. & Pradhan, G.D. 1975. Entomological evaluation of fenitrothion (OMS-43) as a residual spray for the control of <u>An. gambiae</u> and <u>An. funestus</u>, Kisumu, Kenya. WHO/VBC75.547: 1-22.

Fontaine, R.E., Pull, J.H., Payne, D., Pradhan, G.D., Joshi, G.P., Pearson, J.A., Thymakis, M.K. & Camacho, M.E.R. 1978. Evaluation of fenitrothion for the control of malaria. Bull. WHO 56: 445-452.

Foy, H., Kondi, A., Damkas, C., Depanian, M., Lefcopoulou, T., Bach, L.G., Dax, R., Pitchford, J., Shiele, P. & Langton, M. 1948. Malaria and blackwater fever in Macedonia and Thrace in relation to DDT. Ann. Trop. Med. Parasitol. 42: 153-172.

Frederickson, C. 1985. Progress report to the Pan American Health Organization. 9 pp.

Gaaboub, I.A. & Dawood, M.R. 1974. Irritability status of adults of Culex pipiens L. under selection pressure with lethal concentrations of DDT and malathion. Zeitschrift fur Angewandte Entomologie 77: 126-132.

Gaaboub, I.A., El Sawaf, S.K. & El Latif, M.A. 1970. Irritability of adults of Anopheles (Myzomyia) pharoensis Theobald (Dipt. Nem.) at the presence of DDT. Zeitschrift fur Angewandte Entomologie 66: 428-434.

Gabaldon, A. & Berti, A.L. 1954. The first large area in the tropical zone to report malaria eradication: north-central Venezuela. Am. J. Trop. Med. Hyg. 3: 793-807.

Gabaldon, A. 1949. The nation-wide campaign against malaria in Venezuela. Trans. R. Soc. Trop. 43: 113-160.

Gabaldon, A. 1952. The effect of DDT on the population of anopheline vectors in Venezuela. Rivista di Parassitologia. 13: 27-41.

Gabaldon, A. 1972. Difficulties confronting malaria eradication. Am. J. Trop. Med. Hyg. 21: 634-639.

Gahan, J.B. & Lindquist, A.W. 1945. DDT residual sprays applied in buildings to control Anopheles quadrimaculatus. J. Econ. Entomol. 38: 223-230.

Gahan, J.B., Travis, B.V., Morton, F.A. & Lindquist, A.W. 1945. DDT as a residual-type treatment to control Anopheles quadrimaculatus, practical tests. J. Econ. Entomol. 38: 223-235.

Galun, R. 1979. Responses of blood-sucking arthropods to vertebrate hosts. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N. Y. John Wiley & Sons: 103-115.

Galvao, A.L.A. & Damasceno, R.G. 1947. Alguns dados experimentais sobre a acao do D. D. T. e do piretro contra o Anopheles darlingi. Rev. Serv. Esp. Saude. Publ. 1: 273-292.

Gelfand, H.M. 1955. Anopheles gambiae Giles and Anopheles melas Theobald in a coastal area of Liberia, West Africa. Trans. R. Soc. Trop. 49: 508-27.

Georghiou, G.P. & Taylor, C.E. 1977. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Entomol. 70: 319-323.

Georghiou, G.P. (Consultant, USAID) 1990. An analysis of insecticide resistance in malaria vector mosquitoes in Pakistan and recommendations for future action. Islamabad, Pakistan. 51 pp.

Georghiou, G.P. 1972. The evolution of resistance to pesticides. Ann. Rev. Econ. Entomol. 133-168.

Georghiou, G.P. 1980. Insecticide resistance and prospects for its management. Res. Rev. 76: 131-145.

Gerberg, E.J. 1966. Field and laboratory repellency tests with 2, 2, 4-trimethyl-1, 3-pentanediol (TMPD). J. Econ. Entomol. 59: 872-875.

Gerold, J. L. & Laarman, J. J. 1968. Behavioural responses to contact with DDT in Anopheles atroparvus. Acta Leiden. 36: 88-92.

Gerold, J.L. & Laarman, J.J. 1964. Selection of some strains of Anopheles atroparvus with different behavioral responses to contacts with DDT. Nature 204: 500-501.

Gerold, J.L. 1968. DDT irritability in Anopheles atroparvus. Acta Leiden. 36: 86-87.

Gerold, J.L. 1969. Selection of strains of Anopheles atroparvus with different behavior after contact with DDT. Cah. Orstrom. Ser. Entomol. Med. Parasitol. 7: 13-21.

Gerold, J.L. 1977. Behavioural differences in field populations of Anopheles sacharovi in Turkey and in selected strains of Anopheles atroparvus. Acta Leiden. 45: 67-77.

Gerold, J.L. 1977. Evaluation of some parameters of house-leaving behavior of Anopheles gambiae S. L. Acta Leiden. 45: 79-90.

Gheorghiu, T., Ungureanu, E.M. & Garrett-Jones, C. 1971. An apparatus for the study of the behaviour pattern of mosquitos under the influence of insecticides. WHO/VBC. 71.278: 1-8.

Giglioli, G. & Charles, L.J. 1954. Reappearance of Anopheles darlingi Root in a controlled area of British Guiana's coastlands. Am. J. Trop. Med. Hyg. 3: 808-816.

Giglioli, G. 1948. An investigation of the house-frequenting habits of mosquitoes of the British Guiana coastland in relation to the use of DDT. Am. J. Trop. Med. 28: 43-70.

Giglioli, G. 1951. Eradication of Anopheles darlingi from the inhabited areas of British Guiana by DDT residual spraying. J. Nat. Malar. Soc. 10: 142-161.

Giglioli, G. 1956. Biological variations in Anopheles darlingi and Anopheles gambiae: their effect on practical malaria control in the neotropical region. Bull. WHO 15: 461-471.

Gilbert, I. H. 1966. Evaluation and use of mosquito repellents. J. Am. Med. Assoc. 196: 163-165.

Gillies, M.T. & Smith, A. 1960. The effect of a residual house-spraying campaign in East Africa on species balance in the Anopheles funestus group. The replacement of An. funestus Giles by Anopheles rivulorum Leeson. Bull. Entomol. Res. 51: 243-252.

Gillies, M.T. 1956. The problem of exophily in Anopheles gambiae. Bull. WHO 15: 437-449.

Gillies, M.T. 1957. Age-groups and the biting cycle in Anopheles gambiae. A preliminary investigation. Bull. Entomol. Res. 48: 553-559.

Gillies, M.T. 1964. Selection for host preference in Anopheles gambiae. Nature 203: 852-854.

Gokberk, C. 1961. Anopheles sacharovi (Favre 1903) in Turkey. Mosq. News 21: 101-102.

Grandes, A.E. & Sagrado, E.A. 1988. The susceptibility of mosquitoes to insecticides in Salamanca province, Spain. J. Am. Mosq. Control Assoc. 4: 168-171.

Granett, P. 1940. Studies of mosquito repellents, 1. Test procedure and method of evaluating test data. J. Econ. Entomol. 33: 563-65.

Grothaus, R.H. & Adams, J. F. 1972. An innovation in mosquito-borne disease protection. Military Med. 181-184.

Grothaus, R.H., Gouck, H.K., Weidhaas, D.E. & Jackson, S.C. 1974. Wide-mesh netting, an improved method of protection against blood-feeding Diptera. Am. J. Trop. Med. Hyg. 23: 533-537.

Grothaus, R.H., Hirst, J.M., Gouck, H.K. & Weidhaas, D.E. 1972. Field tests with repellent-treated wide-mesh netting against mixed mosquito populations. J. Med. Entomol. 9: 149-152.

Grothaus, R.H., Reed, J.T. & Passingham, L.H. 1976. Field evaluation of arthropod repellent netting as a ground cloth against ticks. Am. J. Trop. Med. Hyg. 25: 747-750.

Gupta, R.K., Rutledge, L.C., Reifenrath, W.G., Gutierrez, G.A. & Korte, D.W. 1989. Effects of weathering on fabrics treated with permethrin for protection against mosquitoes. J. Am. Mosq. Control Assoc. 5: 176-179.

Hadaway, A.B., Barlow, F., Grose, J.E.H., Turner, C.R. & Flower, L.S. 1970. Evaluation of compounds for insecticidal activity on adult mosquitoes. Part 1: Response of adult mosquitoes to some new insecticides. Bull. WHO 42: 353-368.

Haddow, A.J. 1942. The mosquito fauna and climate of native huts at Kisumu, Kenya. Bull. Entomol. Res. 33: 91-142.

Hadjinicolaou, J. & Betzios, B. 1973. Resurgence of Anopheles sacharovi following malaria eradication. Bull. WHO 48: 699-703.

Hall, S.A., Green, N. & Beroza, M. 1957. Insect repellents and attractants. Agricultural and Food Chemistry 5: 663-669.

Hamon, J. 1963. L' importance des changements de comportement chez les insectes [Importance of behavior changes in insects]. Bull. WHO 29(suppl.): 115-120.

Hamon, J., Mouchet, J., Brengues, J. & Chauvet, G. 1970. Problems facing anopheline vector control. Vector ecology and behavior before, during, and after application of control measures. Misc. Publ. Entomol. Soc. Am. 7: 28-44.

Haridi, A.M. 1972. Partial exophily of Anopheles gambiae species B in the Khashm Elgirba area in eastern Sudan. Bull. WHO 46: 39-46.

Hayes, W.J.Jr. 1971. The degree of hazard to man of DDT as used against malaria. WHO/VBC 71.738: 1-17.

Haynes, K.F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Ann. Rev. Entomol. 33: 149-158.

Hecht, O. & Corzo, J.H. 1957. Beitrag zur biologie einiger Mexikanischer anophelen. Zeitschrift fur Tropenmedizin und Parasitologie. 8: 391-403.

Hecht, O. & Hernandez, J. 1960. Resena de las investigaciones sobre la irritacion de anofelinos por el contacto con superficies cubiertas con DDT. CNEP Boletin. 4: 93-106.

Hecht, O., Barrera, A. & Mancera, O. 1958. Quantitative measurements towards an understanding of behavioristic resistance. In: WHO PAHO Sem. on Suscept. Insects to Insectic. 26: 304-317.

Hecth, O., Chapa-Saldana, H. & Hernandez-Corzo. 1962. The influence of illumination upon the direction of the escape flights of anophelines irritated by DDT. Verhanl 11 Kongr. Entomol. Wien, 1960. 3: 79-81.

Hemingway, J. 1983. Biochemical studies on malathion resistance in Anopheles arabiensis from Sudan. Trans. R. Soc. Trop. 77: 477-480.

Hemingway, J. 1989. A note on simple biochemical methods for resistance detection and their field application in Sri Lanka. Pest. Science. 27: 281-285.

Herath, P.R.J. & Joshi, G.P. 1989. Pesticide selection pressure on Anopheles subpictus in Sri Lanka: comparison with two other Sri Lankan anophelines. Trans. R. Soc. Trop. 83: 565-567.

Herath, P.R.J., Hemingway, J., Weerasinghe, I.S. & Jayawardena, K.G.I. 1987. The detection and characterization of malathion resistance in field populations of Anopheles culicifacies B in Sri Lanka. Pest. Biochem. Physiol. 29: 157-162.

Hervy, J.P. & Sales, S. 1982. Stage 4 evaluation of the effectiveness of an insecticide, OMS-2002, on adult Anopheles at Soumousso experimental station (Upper Volta) in two concentrations (0.5 g/m and 0.25 g/m) and using two types of intradomiciliary spraying (total and selective). WHO/VBC82.862: 1-13.

Hervy, J.P., Legros, F. & Sales, S. 1982. Stage 4 evaluation of the effectiveness of two insecticides (OMS-2002 and OMS-2004) on adult Anopheles by intradomiciliary spraying at Soumousso experimental station (Upper Volta) using OMS-1 as the reference product in full or selective treatment. WHO/VBC.82.861: 1-16.

Hess, A.D. 1952. The significance of insecticide resistance in vector control programs. Am. J. Trop. Med. Hyg. 3: 371-388.

Hii, J. L.K. 1979. Entomological evaluation of sumithion, DDT, and malathion for the control of Anopheles balabacensis balabacensis Baisas in Kuala Penyu district, Sabah, Malaysia. Jap. J. Sanit. Zool. 30: 319-28.

Hii, J. L.K. 1985. Evidence for the existence of genetic variability in the tendency of Anopheles balabacensis to rest in houses and to bite man. Southeast Asian J. Trop. Med. Publ. Hlth. 16: 173-182.

Hii, J.L.K. 1984. Insecticide susceptibility studies of three cryptic species of the Anopheles balabacensis complex. Southeast Asian J. Trop. Med. Publ. Hlth. 15: 104-111.

Hirsch, J. 1963. Behavior genetics and individuality understood. Science 142: 1436-1442.

Hocking, B. 1963. The use of attractants and repellents in vector control. Bull. WHO 29(suppl): 121-126.

Hoffman, C.C. 1934. Contribucion al conocimiento del paludismo en la peninsula de Yucatan. Boletin del Instituto de Hygiene Mexico 2: 1-2.

Hooper, G.H.S. & Brown, A.W.A. 1965. A case of developed irritability to insecticides. Bull. WHO 32: 131-132.

Hooper, G.H.S. & Brown, A.W.A. 1965. Development of increased irritability to insecticides due to decreased detoxification. Entomologia Experimentalis et Applicata. 8: 263-70.

Hoskins, W.M. & Lincicome, D.R. 1963. Ed. Resistance to insecticides. International Rev. Trop. Med. 2: 119-174.

Hossain, M.I. & Curtis, C.F. 1989. Laboratory evaluation of deet and permethrin impregnated wide-mesh netting against mosquitoes. Entomologia Experimentalis et Applicata. 52: 93-102.

Hu, S.M.K. 1940. The house-frequenting behavior of Anopheles hyrcanus var. sinensis wiedemann in the Shanghai area, part 3. Indoor resting places. Lingnan Sci. J. 19: 403-410.

Hudson, J.E. & Esozed, S. 1971. The effects of smoke from mosquito coils on Anopheles gambiae Giles and Mansonia uniformis (Theo) in verandah-trap huts at Magugu, Tanzania. Bull. Entomol. Res. 61: 247-265.

Hudson, J.E. 1971. Assessment of methoxy-DDT as a residual spray against mosquitoes in verandah-trap huts at Magugu, Tanzania. Bull. Entomol. Res. 60: 615-630.

Ichimori, K. 1989. Mosquito susceptibility to malaria. Jap. J. Sanit. Zool. 40: 1-12.

Iftner, D.C. & Hall, F.R. 1983. Effects of fenvalerate and permethrin on Tetranychus urticae Koch (Acari: Tetranychidae) dispersal behavior. Environ. Entomol. 12: 1782-1786.

Ikemoto, T. 1978. Studies on the spatial distribution pattern of larvae of the mosquito, Anopheles sinensis, in rice fields. Res. Popul. Ecol. 18: 237-249.

Ismail, A.H., Notananda, V. & Schepens, J. 1975. Studies on malaria and responses of Anopheles balabacensis balabacensis and Anopheles minimus to DDT residual spraying in Thailand; part 2: post- spraying observations. WHO/VBC75.515: 1-24.

Ismail, I.A.H., Phinichpongse, S. & Boonrasri, P. 1978. Responses of Anopheles minimus to DDT residual spraying in a cleared forested foothill area in central Thailand. Acta. Trop. (Basel) 35: 69-82.

Iyengar, M.O.T. 1954. Vector of malaria in Kabul, Afghanistan. Trans. R. Soc. Trop. 48: 319-324.

Jany, W.C. 1903. A review of the factors affecting the use of malathion insecticide in the malaria control project in Pakistan for: USAID, 26 pp.

Jeffery, G.M. 1976. Malaria control in the twentieth century. Am. J. Trop. Med. Hyg. 25: 361-371.

Jinjiang, X., Meiluan, Z., Xinfu, L., Rongen, G., Shixian, P. & Shuyou, L. 1988. Evaluation of permethrin-impregnated mosquito-nets against mosquitoes in China. WHO/VBC. 88.962: 9 pp.

Johnson, H.L., Skinner, W.A., Maibach, H.I., Pearson, T.R. 1967. Repellent activity and physical properties of ring-substituted N, N-diethylbenzamides. J. Econ. Entomol. 60: 173-176.

Jones, G.D.G. & Sylvester, N.K. 1966. Pyrethrum as an insect repellent, part 1: literature review. Pyrethrum. Post. 8: 38-41.

Joshi, G.P., Self, L.S., Usman, S., Pant, C.P., Nelson, M.J. & Supalin. 1977. Ecological studies on Anopheles aconitus in the Semarang area of central Java, Indonesia. WHO/VBC77.677: 1-15.

Kaissling, K.E. 1979. Control of insect behavior via chemoreceptor organs. In: Shorey, H. H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N.Y. John Wiley & Sons; 45-65.

Kalmus, H. & Hocking, B. 1960. Behavior of Aedes mosquitoes in relation to blood-feeding and repellents. Entomologia Experimentalis et Applicata. 3: 1-26.

Kaschef, A.H. 1970. Effects of temperature on the irritability caused by DDT and DDT-analogues in anopheline mosquitoes. Bull. WHO 42: 917-930.

Kay, B.H. 1982. Investigation & control of vectors of arthropod-borne diseases. Australian Institute of Health Surveyors 43: 70-72.

Kay, B.H. 1985. Man-mosquito contact at Kowanyama, Northern Queensland, Australia. J. Am. Mosq. Control Assoc. 1: 191-194.

Kenawy, M.A., Beier, J.C. & El Said, S. 1986. First record of malaria and associated Anopheles in El Gara Oasis, Egypt. J. Am. Mosq. Control Assoc. 2: 101-103.

Kennedy, J.S. 1947. The excitant and repellent effects on mosquitoes of sub-lethal contacts with DDT. Bull. Entomol. Res. 37: 593-607.

Kennedy, J.S. 1979. Behaviorally discriminating assays of attractants and repellents. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N. Y. John Wiley & Sons: 215-229.

Kennedy, J.S. 1979. Olfactory responses to distant plants and other sources. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N.Y. John Wiley & Sons: 67-91.

Kerr, J.A. 1964. Malaria eradication in the Americas-- operational research. Am. Rev. World. Hlth.1: 11-13.

Khan, A.A. 1979. Mosquito attractants and repellents. In: Shorey, H.H. & McKelvey, J. J., editors. Chemical control of insect behavior: theory and application. New York, N. Y., John Wiley & Sons: 305-325.

Khan, A.A., Maibach, H.I. & Skidmore, D.L. 1973. A study of insect repellents. 2. Effect of temperature on protection time. J. Econ. Entomol. 66: 437-438.

Khan, A.A., Maibach, H.I. & Skidmore, D.L. 1975. Addition of perfume fixatives to mosquito repellents to increase protection time. Mosq. News 35: 23-26.

King, W.V. & Gahan, J.B. 1949. Failure of DDT to control house flies. J. Econ. Entomol. 42: 405-409.

King, W.V. 1948. Some results of recent work on the newer insecticides. Am. J. Trop. Med. 28: 487-497.

Kirnowardoyo, S. 1988. Anopheles malaria vector and control measures applied in Indonesia. Southeast Asian J. Trop. Med. Publ. Hlth. 19: 713-716.

Knipling, E.F. 1952. Present status of mosquito resistance to insecticides. Am. J. Trop. Med. Hyg. 3: 389-394.

Kondrashin, A.V. 1987. Factors determining the dynamics of malaria systems in South Asia. J. Commun. Dis. 19: 75-80.

Kouznetsov, R.L. 1976. Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on population health. WHO/MAL76.881: 1-16.

Kring, J.B. 1958. Feeding behavior and DDT resistance of Epitrix cucumeris (Harris). J. Econ. Entomol. 51: 823-828.

Kuhlow, F. & Garms, R. 1964. Laboratoriumsuntersuchungen uber die irritabilitat einer naturlichen population von Anopheles atroparvus gegenuber DDT. Proc. 12th Int. Congr. Entomol: 12: 807-809.

Kuhlow, F. 1959. On the behavior of Anopheles funestus in unsprayed and DDT sprayed houses. Zeitschrift fur Tropenmedizin und Parasitologie 10: 328-329.

Kuhlow, F. 1962. Field experiments on the behavior of malaria vectors in an unsprayed hut and in a hut sprayed with DDT in northern Nigeria. Bull. WHO 26: 93-102.

Kulkarni, S.M. 1987. Feeding behavior of anopheline mosquitoes in an area endemic for malaria in Bastar district, Madhya Pradesh. Ind. J. Malariol. 24: 163-171.

Kurihara, T. & Umino, T. 1987. The effects of insecticides on the behavior of female mosquitoes under laboratory conditions. Jap. J. Sanit. Zool. 38: 25-32.

Laarman, J.J. 1958. The host-seeking behavior of anopheline mosquitoes. Trop. Geogr. Med.10: 293-305.

Lal, H., Ginocchio, S. & Hawrylewicz, E. J. 1965. Effect of allethrin on feeding behavior of insects. Proc. Soc. Exp. Biol. Med. 120: 441-443.

Languillon, J., Mouchet, J., Rivola, E. & Rateau, J. 1956. Contribution a l'etude de l'epidemiologie du paludisme dans la region forestiere du Cameroun. Med. Tropicale. 16: 347-362.

Lavagnino, A. 1983. Anopheles labranchiae in Sicily: decrease of susceptibility to insecticides. Trans. R. Soc. Trop.77: 883.

Lee, L.G. 1957. Preliminary observation on the effect of DDT indoor treatment on Anopheles hyrcanus var.sinensis and Anopheles jeyporiensis var. candidiensis. Acta. Entomologica Sinica. 7: 481-488.

Lewi, P.J. 1980. Multivariate data analysis in structure-activity relationships. In: Ariens, E. J., editor. Drug Design. New York: Academic Press. 10: 307-342.

Linduska, J.P. & Morton, F.A. 1947. Determining the repellency of solid chemicals to mosquitoes. J. Econ. Entomol. 40: 562-564.

Lines, J.D. & Curtis, C.F. 1984. Attempts to assess the protection conferred by DDT resistance in An. gambiae. Mosquito studies at the London School of Hygiene and Tropical Medicine, Progress report #45. London School of Hygiene and Tropical Medicine. London 17-18.

Lines, J.D., Curtis, C.F., Myamba, J. & Njau, R. 1985. Tests of repellents or insecticide impregnated curtains, bednets and anklets against malaria vectors in Tanzania. WHO/VBC85.920: 1-16.

Lockwood, J.A., Sparks, T.C. & Story, R.N. 1984. Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30: 41-51.

Loong, K.P., Naidu, S., Thevasagayam, E.S. & Cheong, W.H. 1985. Evaluation of the effectiveness of permethrin and DDT impregnated bed-nets against Anopheles maculatus. Southeast Asian J. Trop. Med. Publ. Hlth 16: 554-559.

Lu, B. 1988. The effect of Azolla on mosquito breeding. Parasitol. Today 11: 328-329.

Ludvik, G.F., Snow, W.E. & Hawkins, W.B. 1951. The susceptibility of Anopheles quadrimaculatus to DDT after five years of routine treatment in the Tennessee River Valley. J. Nat. Malar. Soc.10: 35-43.

Luh, P.L., Kao, C.T., Chang, P.C. & Liu, C.Y. 1963. Studies on the behavior of Anopheles sinensis Wiedemann, 1828, A. jeyporiensis candidiensis Koidzumi, 1924, and Anopheles minimus Theobald, 1901 in DDT and BHC treated huts. Acta Entomologica Sinica. 12: 149-162.

MacCormack, C.P., Snow, R.W. & Greenwood, B.M. 1989. Use of insecticide-impregnated bed nets in Gambian primary health care: economic aspects. Bull. WHO 67: 209-214.

MacDonald, G. 1950. The economic importance of malaria in Africa. WHO/Mal/Conf 16: 1-17 pp.

Mahadevan, B. & Varma, R.N. 1967. Laboratory evaluation of certain repellents against mosquitoes. Armed Forces Med. J. India. 23: 195-201.

Mahmood, F. & Macdonald, M.B. 1985. Ecology of malaria transmission and vectorial capacity of Anopheles culicifacies species A in rural Punjab, Pakistan. Pakistan J. Med. Res. 24: 95-106.

Maibach, H.I., Skinner, W.A., Strauss, W.G. & Khan, A.A. 1966. Factors that attract and repel mosquitoes in human skin. J. Am. Med. Assoc. 196: 263-266.

Maibach, H.I., Strauss, W.G. & Khan, A.A. 1969. Attractiveness and repellency of man to mosquito bites. Ann. Prog. Rep. US Army Med R & D Command, Office of the Surgeon General. Washington, D.C.

Malcolm, C.A. 1988. Reduced susceptibility to permethrin and its relationship to DDT resistance in larvae of Anopheles stephensi. Med. Vet. Entomol. 2: 37-46.

Mancera, O. & Hernandez, J. 1960. El efecto irritante en mosquitos de algunos insecticidas de contacto. CNEP Boletin. 4: 82-85.

Mariani, M., Marceca, G., Oddo, F. & Terminello, L. 1959. Ulteriori indaginisulla sensibilita' al DDT dell' Anopheles labranchiae in Sicilia. Rivista di Parassitologia 20: 191-198.

Martinez-Palacios, A. & de Zulueta, J. 1964. Ethological changes in Anopheles pseudopunctipennis in Mexico after prolonged use of DDT. Nature 203: 940-941.

Marwoto, H.A. 1987. Malaria studies in Indonesia. Zbl. Bakt. Hyg. 264: 326-332.

Matola, Y.G., White, G.B. & Magayuka, S.A. 1987. The changed pattern of malaria endemicity and transmission at Amani in the eastern Usambara mountains, north-eastern Tanzania. J. Trop. Med. Hyg. 90: 127-134.

Matsushima, T. 1987. Malaria situation in Asia with special reference to western Pacific region. Zbl. Bakt. Hyg. 264: 333-336.

Mattingly, P.F. 1962. Mosquito behaviour in relation to disease eradication programmes. Ann. Rev. Entomol.7: 419-436.

Mattingly, P.F. 1967. Genetics of behavior. In: Genetics of Insect Vectors of Disease. Eds Wright, J.W. et al. Elsevier Publishing, New York, 533-566.

Maxwell, F.G. 1979. Host-plant resistance to insects--chemical relationships. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N.Y. John Wiley & Sons; 299-304.

Mazza, S. & Rickard, E. 1930. Relacion del cultivo de arroz con la difusion del paludismo en la provincia de Tucuman (informe sobre estudios en una estacion completa). Reunion de la Sociedad Argentina de Patologia Regional del Norte, Argentina. 707-711.

McClelland, G.A.H. 1973. Some man-made mosquito problems in Africa and prospects for their rational solution. Tall Timbers Conference on Ecological Animal Control by Habitat Management 5: 27-41.

McCray, E.M. & Schoof, H.F. 1970. Laboratory behavior of Culex pipiens quinquefasciatus and the effects of tepa, metepa, and apholate upon its reproduction. Mosq. News 30: 149-155.

McDonald, J.L. & Grothaus, R.H. 1973. Field studies using wide-mesh mosquito bed nets in Taiwan and Indonesia. J. Med. Entomol. 10: 299.

McGovern, T.P., Beroza, M. & Gouck, H. 1967. Chemicals tested as space repellents for yellow-fever mosquitoes. 2. Carbanilates, benzamides, aliphatic amides, and imides. J. Econ. Entomol. 60: 1591-1594.

McGovern, T.P., Schreck, C.E. & Jackson, J. 1984. Mosquito repellents: N, N-dimethylbenzamides, N, N-dimethylbenzeneacetamides, and other selected N, N-dimethylcarboxamides as repellents for Aedes aegypti, Anopheles quadrimaculatus and Anopheles albimanus. Mosq. News 44: 11-16.

Meek, S.R. 1988. Epidemiology of malaria in displaced Khmers on the Thai-Kampuchean border. Southeast. Asian. J. Trop. Med. Publ. Hlth. 19: 243-52.

Milby, M.M. & Reisen, W.K. 1989. Estimation of vectorial capacity: vector survivorship. Bull. Soc. Vector Ecol. 14: 47-54.

Miller, L.H. 1992. The challenge of malaria. Science 257: 36-37.

Mills, A. J. 1987. Economic study of malaria in Nepal: the cost effectiveness of malaria control strategies. (Evaluation and Planning Centre, London School of Hyg and Trop Med). 139 pp.

Ministerio de agricultura y ganaderia, direccion general de sanidad vegetal. Informe anual departamento de abonos y plaguicidas, 1987. PAHO Regional Conference. 1988: 24 pp.

Moore, C.G.1977. Insecticide avoidance by ovipositing Aedes aegypti. Mosq. News 37: 291-293.

Moray, N. & Connolly, K.A 1963. Possible case of genetic assimilation of behavior. Nature 199: 358-360.

Morris, K.R.S. Problems in the assessment of tsetse populations. Bull. Entomol. Res. 239-256.

Mouchet, J. & Cavalie, P. 1961. L'irritabilite vis a vis du DDT d' Anopheles gambiae et d' A. funestus dans le Nord-Cameroun. Rivista di Malariologia 40: 191-217.

Mouchet, J. & Hamon, J. 1963. Difficulties in malaria eradication campaigns due to the behavior of the vectors. WHO/VBC 16: 1-11.

Mouchet, J.; Hamon, J. 1963. Les problemes techniques de l'eradication du paludisme en Afrique. Cah. Orstrom Ser. Entomol. Med. Parasitol. 1: 39-48.

Moulton, F.R. 1941. A Symposium on Human Malaria with Special Reference to North America and the Caribbean Region. American Association for the Advancement of Science, Washington, D.C. 398 pp.

Muir, D.A. 1975. Genetic aspects of developing insecticide resistance of malaria vectors; part 1. Selection pressures. WHO/VBC75.571: 1-14.

Muirhead-Thomson, R.C. & Mercier, E.C. 1952. Factors in malaria transmission by Anopheles albimanus in Jamaica, part 1. Ann. Trop. Med. Parasitol. 46: 103-116.

Muirhead-Thomson, R.C. 1949. DDT and 'gammexane' as residual insecticides against Anopheles gambiae in African houses. Nature 163: 109-110.

Muirhead-Thomson, R.C. 1951. Mosquito Behavior in Relation to Malaria Transmission and Control in the Tropics. Edward Arnold & Co., London. 219 pp.

Muirhead-Thomson, R.C. 1960. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bull. WHO 22: 721-34.

Muirhead-Thomson, R.C., Gordon, R.M. & Davey, T.H. 1952. A plea for the standardization of experimental work on residual insecticides. Trans. R. Soc. Trop. 46: 271-274.

Mulla, M.S., Mian, L.S. & Gratz, N.G. 1987. Agricultural management practices-their impacts on production of vector and pest mosquitoes. J. Agric. Entomol. 4: 97-131.

Mulligan, H.W. & Baily, J.D. 1936. Malaria in Quetta, Baluchistan. Rec. Mal. Sur. India 6: 289-295.

Munakata, K. 1979. Insect feeding deterrents in plants. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N.Y. John Wiley & Sons 93-102.

Najera-Morrondo, J.A. 1979. A suggested approach to malaria control and to the methodology applicable in different epidemiologic situations, based on experience in the Americas. Bull. PAHO 13: 223-234.

Najera-Morrondo, J.A. 1989. Malaria and the work of World Health Organization. Bull. WHO 67: 229-243.

Nolan, J. 1985. Mechanisms of resistance to chemicals in arthropod parasites of veterinary importance. Vet. Parasitol. 18: 155-166.

Nutsathapana, S., Sawasdiwongphorn, P., Chitprarop, U. & Cullen, J.R. 1986. The behaviour of Anopheles minimus Theobald (Diptera: Culicidae) subjected to differing levels of DDT selection pressure in northern Thailand. Bull. Entomol. Res. 76: 313-320.

O' Connor, C.T. & Arwati 1974. Insecticide resistance in Indonesia. WHO/VBC74.505: 1-8.

Ohoa, J.O., Molina, P.A. & Calderon, M.L. 1988. Breve resumen de los insecticidas agricola en la resistencia del Anopheles albimanus en Guatemala. PAHO Regional Conf. 3 pp.

Onori, E., Nushin, M.K., Cullen, J.E., Yakubi, G.H., Mohammed, K. & Christal, F.A. 1975. An epidemiological assessment of the residual effect of DDT on Anopheles hyrcanus sensulato and A. pulcherrimus (Theobold) in the northeastern region of Afghanistan. Trans. R. Soc. Trop. 69: 236-242.

Ossi, G.T. 1985. Malaria in Iraq for the years 1984 and 1985. Malaria Coordination Meeting: Institute of Endemic Diseases, Baghdad. Ankara, Turkey: 5-20.

Pal, R. 1964. Methods for studying the behavior of malaria vectors under the impact of residual insecticides. WHO/VBC89.64: 1-10.

Pant, C. 1987. Vector-borne diseases and their socio-economic impact. Insect Sci. Appl. 8: 655-664.

Pant, C.P., Rishikesh, N., Bang, Y.H. & Smith, A. 1981. Progress in malaria vector control. Bull. WHO 59: 325-333.

Peele, D.B. & Crofton, K.M. 1987. Pyrethroid effects on schedule-controlled behavior: time and dosage relationships. Neurotoxicology and Teratology 9: 387-394.

Perkins, J.H. 1978. Reshaping technology in wartime: the effect of military goals on entomological research and insect-control practices. 169-186.

Peters, W. & Standfast, H.A. 1960. Studies on the epidemiology of malaria in New Guinea: part 2. Holoendemic malaria- the entomological picture. Trans. R.Soc. Trop. 54: 249-254.

Peters, W. 1960. Studies on epidemiology of malaria in New Guinea: part 3. Holoendemic malaria-analysis of data and relation to control. Trans. R. Soc. Trop. 54: 254-260.

Pinotti, M. 1951. The nationwide malaria eradication program in Brazil. J. Nat. Malar. Soc. 10: 162-182.

Pinotti, M., Rachou, R.G. & Ferreira, M.O. 1947. Algunos aspectos epidemiologicos de la malaria en el litoral sur del Brasil en la zona de transmision por anofelinos del subgenero Kerteszia. Tijeretazos sobre Malaria 11: 1-25.

Pluthero, F.G. & Singh, R.S. 1984. Insect behavioral responses to toxins: practical and evolutionary considerations. Can. Entomol. 116: 57-68.

Pluthero, F.G. & Threlkeld, S.F.H. 1981. Genetic differences in malathion avoidance and resistance in Drosophila melanogaster. J. Econ. Entomol. 74: 736-740.

Pradhan, S.P. 1986 (USAID/Nepal). [Letter to Calder, D. H.]. 1986 Apr 15. 8 pages.

Prasittisuk, C. 1985. Present status of malaria in Thailand. Southeast Asian J. Trop. Med. Publ. Hlth. 16: 141-145.

Quinones, M.L. & Suarez, M.F. 1989. Irritability to DDT of natural populations of the primary malaria vectors in Colombia. J. Am. Mosq. Control Assoc. 5: 56-59.

Qutubuddin, M. 1967. Irritability of Anopheles pharoensis Theobald to different insecticides as observed in laboratory experiments. Sudan Med. J. 5: 18-28.

Quy, D.V., Ai, N. V. & Vandekerkove, M. 1963. Apercu sur le comportement trophique des Anopheles au Sud-Viet-nam [Note on the trophic behavior of Anopheles in South Vietnam]. Bull. Soc. Pathol. Exot. 56: 305-309.

Rachou, R.G. 1958. Anofelinos do Brasil: comportamento das especies vetoras de malaria. Rev. Bras. Malariol. Doencas Trop. 10: 145-181.

Rachou, R.G. 1958. Some manifestations of behavioristic resistance in Brazil. In: WHO PAHO Sem. on Suscept. Insects to Insectic. 280-294.

Rachou, R.G. 1959. Suscetibilidade de anofelinos Brasileiros a inseticidas clorados. Resultados de sua determinacao ate 1959 [Susceptibility of Brazilian anophelines to chlorinated insecticides. Results of its determination up to 1959]. Rev. Bras. Malariol. Doencas. Trop. 12: 59-68.

Rachou, R.G., Ferreira, M.O., Lobo, A.G.S. & Pires, A.M. 1954. Consideracoes gerais sobre a epidemiologia da malaria no sul do Brasil. Rev. Bras. Malariol. Doencas. Trop. 6: 177-188.

Rachou, R.G., Lobo, A.G.S. & Luz, E. 1954. Dispersao do Anopheles darlingi no recrudescimento epidemico de malaria em 1950 no norte do Parana. Rev. Bras. Malariol. Doencas Trop. 6: 411-414.

Rachou, R.G., Lyons, G., Moura-Lima, M. & Kerr, J.A. 1965. Synoptic epidemiological studies of malaria in El Salvador. Am. J. Trop. Med. Hyg. 14: 1-62.

Rachou, R.G., Moura Lima, M., Paulini, E. & Memoria, J.M.P. 1957. Primeiras provas de determinacao da susceptibilidade de anofelinos Brasileiros aos inseticidas. 2: Anopheles (nyssorhynchus) darlingi, em Engenheiro Dolabela (estado de Minas Gerais). Rev. Bras. Malariol. Doencas. Trop. 9: 351-359.

Rachou, R.G., Moura-Lima, M., Duret, J. P. & Kerr, J. A. 1963. Experiences with the excito-repellency test box-- model OPS. Proc of the 50th Annu Meet of N. J. Mosq. Exter. Assoc. and the 19th Ann. Meet of Am. Mosq. Control Assoc. 442-447.

Rachou, R.G., Ricciardi, I., Luz, E. & Garbelini, J. 1949. Observacoes, no estado do Parana, sobre o pouso dos anofelinos, vetores de malaria no interior das casas. Rev. Bras. Malariol. Doencas Trop. 1: 288-298.

Rachou, R.G., Schinazi, L.A. & Moura-Lima, M. 1966. Preliminary note on the epidemiological studies made in El Salvador to determine the causes of the failure of residual spraying to interrupt the transmission of malaria. Rev. Bras. Malariol. Doencas Trop. 18: 763-779.

Rafi, S.M. 1955. Resting places inside houses of A. culicifacies and A. stephensi, malaria vectors of the Punjab. Pakistan J. Hlth. 5: 146-154.

Rahman, M., Elias, M. & Ameen, M.U. 1977. Bionomics of Anopheles balabacensis balabacensis Baisas (Diptera: Culicidae) in Bangladesh and its relation to malaria. Bangladesh. J. Zool. 5: 1-23.

Randrianasolo, B.O.R. & Coluzzi, M. 1987. Genetical investigations of zoophilic and exophilic Anopheles arabiensis from Antananarivo area (Madagascar). Parassitologia. 29: 93-97.

Rawlings, P. & Davidson, G. 1982. The dispersal and survival of Anopheles culicifacies Giles (Diptera: Culicidae) in a Sri Lankan village under malathion spraying. Bull. Entomol. Res. 72: 139-144.

Rawlings, P., Herath, P.R.J. & Kelly, S. 1985. Anopheles culicifacies (Diptera: culicidae): DDT resistance in Sri Lanka prior to and after cessation of DDT spraying. J. Med. Entomol. 22: 361-365.

Rayner, H.B. & Wright, R.H. 1966. Far infrared spectra of mosquito repellents. Can Entomol. 98: 76-80.

Ree, H.I. & Loong, K.P. 1989. Irritability of Anopheles farauti, An. maculatus and Culex quinquefasciatus to permethrin. Jap. J. Sanit. Zool. 40: 47-51.

Ree, H.I. 1988. Experimental hut studies on the effect of permethrin-treated mosquito-nets against Anopheles farauti in the Solomon Islands. WHO/VBC88.963: 1-7.

Rehn, J.W.H., Maldonado, C.J. & Henderson, J.M. 1950. Field studies on the bionomics of Anopheles albimanus. Parts 2 and 3: diurnal resting places-progress report. J. Nat. Mal. Soc. 9: 268-279.

Renjifo, S. & de Zulueta, J. 1952. Five years' observations of rural malaria in Eastern Colombia. Am. J. Trop. Med. Hyg.1: 598-611.

Rettich, F. 1980. Residual toxicity of wall-sprayed organophosphates, carbamates and pyrethroids to mosquito Culex pipiens molestus Forskal. J. Hyg. Epi. Microbiol. Immunol. 24: 110-17.

Rishikesh, N., Clarke, J.L., Mathis, H.L., Pearson, J. & Obanewa, S.J. 1979. Stage 5 field evaluation of decamethrin against Anopheles gambiae and Anopheles funestus in a group of villages in Nigeria. WHO/VBC79.712: 1-25.

Rishikesh, N., DiDeco, M.A., Petrarca, V. & Coluzzi, M. 1985. Seasonal variations in indoor resting Anopheles gambiae and Anopheles arabiensis in Kaduna, Nigeria. Acta. Trop(Basel) 42: 165-170.

Rivera, L.A. & Ortiz, M.A. 1988. Estado actual de la susceptibilidad de los vectores en Honduras 1988. PAHO Regional Conference, 13 pp.

Rivera, P., Lopez, J.D. & Picado, M.R. Revision de la resistancia de los vectores a los insecticidas en Nicaragua en los ultimos 10 anos, 1978-1988. PAHO Regional Conference. 4 pp.

Robert, V., Gazin, P., Boudin, C., Molez, J. F., Ouedraogo, V. & Carnevale, P. 1985. La transmission du paludisme en zone de savane arboree et en zone rizicole des environs de Bobo Dioulasso (Burkina Faso) [Malaria transmission in wooded grassland and in rice field areas around Bobo Dioulasso (Burkina Faso)]. Ann. Soc. Belge. Med. Trop. 65: 201-214.

Roberts, D.R., Alecrim, W.D., Tavares, A.M. & McNeill, K.M. 1984. Influence of physiological condition on the behavioral response of Anopheles darlingi to DDT. Mosq. News 44: 357-362.

Roberts, D.R., Alecrim, W.D., Tavares, A.M. & Radke, M.G. 1987. The house-frequenting, host-seeking and resting behavior of Anopheles darlingi in southeastern Amazonas, Brazil. J. Am. Mosq. Control Assoc. 3: 433-441.

Rojanapremsuk, J., Keittivuti, B., Palasudhi, K. & Mongkolpanya, K. 1988. A study on the environmental management and primary health care approach for the control of malaria vectors in Klaeng district, Rayong province, Thailand. Mosquito-Borne Dis. Bull. 5: 1-8.

Ronnefeld, F. 1957. Beobachtungen uber das verhalten von Anopheles punctimaculatus nach der anwendung von DDT. Zeitschrift fur Tropenmedicin und Parasitologie 8: 378-391.

Rossignol, P.A., Ribeiro, J.M.C., Jungery, M., Turell, M.J., Spielman, A. & Bailey, C. L. 1985. Enhanced mosquito blood-finding success on parasitemic hosts: Evidence for vector-parasite mutualism. Proc. Natl. Acad. Sci. 82: 7725-7727.

Rowland, M.W. 1984. Mosquito behavior as a factor in resistance management. Mosquito studies at the London School of Hygiene and Tropical Medicine, Progress Report #45. London School of Hygiene and Tropical Medicine. London.

Rozendaal, J.A., Van Hoof, J.P.M., Voorham, J. & Oostburg, B.F.J. 1989. Behavioral studies of Anopheles darlingi in Suriname to DDT residues on house walls. J. Am. Mosq. Control Assoc. 5: 339-350.

Ruebush, T.K., Breman, J.G., Kaiser, R.L. & Warren, M. 1986. Selective primary health care: 24. Malaria. Rev. Infect. Dis. 8: 454-466.

Rust, M.K. & Reierson, D.A. 1977. Using pheromone extract to reduce repellency of blatticides. J. Econ. Entomol. 70: 34-38.

Rutledge, L.C. 1988. Some corrections to the record on insect repellents and attractants. J. Am. Mosq. Control Assoc. 4: 414-425.

Rutledge, L.C., Collister, D.M., Meixsell, V.E. & Eisenberg, G.H.G. 1983. Comparative sensitivity of representative mosquitoes (Diptera: Culicidae) to repellents. J. Med. Entomol. 20: 506-510.

Rutledge, L.C., Wirtz, R.A., Buescher, M.D. & Mehr, Z.A. 1985. Mathematical models of the effectiveness and persistence of mosquito repellents. J. Am. Mosq. Control Assoc. 1: 56-62.

Sacca, G. & Guy, Y. 1960. Resistance de comportement au DDT chez A. labranchiae au maroc. Bull. WHO 735-741.

Salazar, N.P., Miranda, M.E.G., Santos, M.N. & Llagas, L.A. 1988. The malaria situation in the Philippines with special reference to mosquito vectors. Southeast Asian J. Trop. Med. Publ. Hlth. 19: 709-12.

Saliternik, Z. 1955. The specific biological characteristics of Anopheles (Myzomyia) sergentii (Theo.) and their correlation with malaria control in Israel. Bull. Entomol. Res. 46: 445-462.

Saxena, S.C. & Sumithra, L. 1986. Mosquito repellents from plants. Pestic. 20: 57.

Schofield, C.J. & White, G.B. 1984. Engineering against insect-borne diseases in the domestic environment: house design and domestic vectors of disease. Trans. R. Soc Trop. 78: 285-292.

Schoof, H.F., Mathis, W., Taylor, R.T., Brydon, H.W. & Goodwin, W.J. 1966. Studies with dichlorvos residual fumigant as a malaria eradication technique in Haiti. Am. J. Trop. Med. Hyg. 15: 661-669.

Schoonhoven, L.M. 1979. Insect chemosensory responses to plant and animal hosts. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N.Y. John Wiley & Sons 7-14.

Schreck, C.E. 1977. Techniques for the evaluation of insect repellents: a critical review. Annu. Rev. Entomol. 22: 101-119.

Schreck, C.E. 1985. The status of deet(N, N-diethyl-M-toluamide) as a repellent for Anopheles albimanus. J. Am. Mosq. Control Assoc. 1: 98-100.

Schreck, C.E., Gilbert, I.H., Weidhaas, D.E. & Posey, K.H. 1970. Spatial action of mosquito repellents. J. Econ. Entomol. 63: 1576-1578.

Schreck, C.E., Weidhaas, D.E., Smith, N. & Posey, K.H. 1977. Chemical treatment of wide-mesh net clothing for personal protection against blood-feeding arthropods. Mosq. News 37: 455-462.

Sergieva, V.P., Gracheva, G.V. & Rasnitsin, S.P. 1988. Chuvstvitel 'nost' komarov Anopheles k DDT i malationu v zavisimosti ot pola, vozrasta i pitaniia samok krov'iu [Sensitivity of Anopheles mosquitoes to DDT and malathion depending on sex, age, and female blood feeding]. Med. Parazitol. (Mosk) 4: 27-31.

Service, M.W. 1964. The behavior of Anopheles nili Theo. in sprayed huts in northern Nigeria. J. Trop. Med. Hyg. 67 11-12.

Service, M.W. 1964. The behavior of malaria vectors in huts sprayed with DDT and a mixture of DDT and malathion in northern Nigeria. Trans. R. Soc. Trop. 1: 72-79.

Service, M.W. 1991. Agricultural development and arthropod-borne diseases: a review. Rev. Saude. Publica. 25: 165-178.

Sevilla-Casas, E. 1988. An anthropological approach to the study of human circulation and malaria risk. UNDP/World Bank/WHO/Special Program Research Paper 30: 1-45.

Shalaby, A.M. 1965. Irritability to DDT of certain adult anopheline mosquitoes. J. Egypt Publ. Hlth. Assoc. 40: 283-291.

Shalaby, A.M. 1966. Observations on some responses of Anopheles culicifacies to DDT in experimental huts in Gujarat state, India. Ann. Entomol. Soc. Am. 59: 938-944.

Shalaby, A.M. 1969. Host-preference observations on Anopheles culicifacies (Diptera: culicidae) in Gujarat state, India. Ann. Entomol. Soc. Am. 62: 1270-1273.

Shamrai, A.F. 1969. Specific reaction of mosquitoes to repellents. Parazitologiia. 3: 329-330.

Sharma, V.P. & Mehrotra, K.N. 1986. Malaria resurgence in India: a critical study. Soc. Sci. Med. 22: 835-845.

Sharma, V.P. & Sharma, R.C. 1986. Review of integrated control of malaria in Kheda district, Gujarat, India. Community Participation for Disease Vector Control, Proc. ICMR/WHO 3: 59-84.

Sharma, V.P. & Uprety, H.C. 1982. Preliminary studies on irrigation malaria. Ind. J. Malariol. 19: 139-142.

Sharma, V.P., Uprety, H.C., Nanda, N., Raina, V.K., Parida, S. K. & Gupta, V.K. 1982. Impact of DDT spraying on malaria transmission in villages with resistant Anopheles culicifacies. Indian. J. Malariol. 19: 5-12.

Sharp, B.L., Quicke, F.C. & Jansen, E.J. 1984. Aspects of the behavior of five anopheline species in the endemic malaria area of Natal. J. Entomol. Soc. South. Afr. 47: 251-258.

Shidrawi, G.R. 1970. The man-biting activity of Anopheles gambiae s.1. in DDT-sprayed and unsprayed villages of the dry savanna in Nigeria. WHO/VBC70.197: 1-17.

Shidrawi, G.R. 1989. The VBC computer programme for the global monitoring of vector resistance to pesticides. WHO/VBC/IC-ISR89.27: 1-7.

Shiff, C. 1987. Insecticide impregnated bed-nets and curtains for malaria control. AID/S & T/H/CD 1-5.

Sholdt, L.L., Grothaus, R.H., Schreck, C.E. & Gouck, H.K. 1975. Field studies using repellent-treated wide mesh net jackets against Glossina morsitans in Ethiopia. East. Afr. Med. J. 52: 277-283.

Sholdt, L.L., Holloway, M.L., Chandler, J.A., Fontaine, R.E. & Van Elsen, A. 1977. Dwelling space repellents: their use on military tentage against mosquitoes in Kenya, East Africa. J. Med. Entomol. 14: 252-253.

Sholdt, L.L., Holloway, M.L., Grothaus, R.H. & Schreck, C.E. 1976. Dwelling space repellents: effect upon behavioral responses of mosquitoes in Ethiopia. Mosq. News 36: 327-331.

Shorey, H.H. 1979. Interaction of insects with their chemical environment. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical control of insect behavior: theory and application. New York, N.Y: John Wiley & Sons 1-5.

Simmons, S.W. 1945. Tests on the effectiveness of DDT in anopheline control. Publ. Hlth. Rept. 60: 917-927.

Simonet, D.E., Knausenberger, W.I., Townsend, L.H. Jr. & Turner, E.C.A, 1978. biomonitoring procedure utilizing negative phototaxis of first instar Aedes aegypti. Arch. Environ. Contam. Toxicol. 7: 339-347.

Skinner, W.A. & Johnson, H.L. 1980. The design of insect repellents. In: Ariens, E.J., editor. Drug Design. New York: Academic press. 10: 277-305.

Smatov, Z.H.S. & Kozlova, R.G. 1976. The repellent activity of antikomarin in the conditions of Kazakhstan. Izvestiia Akademii Nauk Kazakhskoi SSR. Seriia Biologicheskirkh. Nauk. 3: 32-35.

Smith, A. & Chabeda, P.I.M. 1969. A verandah trap hut for studying the house-frequenting habits of mosquitoes and for assessing insecticides. The effect of phthalthrin (neopynamin) on the behavior and mortality of Anopheles gambiae Giles. WHO/MAL69.699: 1-13.

Smith, A. & Chabeda, P.I.M. 1969. A verandah-trap hut for studying the house-frequenting habits of mosquitoes and for assessing insecticides. Part 4: The effect of tetramethrin on the behavior and mortality of Anopheles gambiae Giles. Bull. Entomol. Res. 59: 457-463.

Smith, A. & Chadwick, P.R. 1964. A reassessment of pyrethrum against Anopheles gambiae with particular reference to its action as a residual repellent. East Afr. Med. J. 41: 137-141.

Smith, A. & Webley, D.J.A 1969. verandah trap for studying the house-frequenting habits of mosquitoes and for assessing insecticides. Part 3: The effect of DDT on behavior and mortality. Bull. Entomol. Res. 59: 33-46.

Smith, A. 1961. Resting habits of Anopheles gambiae and Anopheles pharoensis in Salt Bush and in crevices in the ground. Nature 190: 1220-1221.

Smith, A. 1962. Effects of dieldrin on the behavior of Anopheles gambiae. Bull. WHO 26: 120-125.

Smith, A. 1963. Assessment of the effect of the insecticide bayer 37344 on the behavior of Anopheles gambiae. Mosq. News 23: 319-321.

Smith, A. 1963. Deterrent effect of insecticides on malaria vectors. Nature 200: 861-862.

Smith, A. 1963. Principles in assessment of insecticides by experimental huts. Nature 198: 171-173.

Smith, A., Obudho, W.O. & Esozed, S. 1966. Resting patterns of Anopheles gambiae in experimental huts treated with malathion. Trans. R. Soc. Trop. 60: 401-408.

Smith, A.A. 1964. Review of the origin and development of experimental hut techniques used in the study of insecticides in East Africa. East Afr. Med. J. 41: 361-374.

Smith, C. N. 1957. (Entomology Research Division, Agricultural Research Service, USDA). Insect repellents. In: 44th Annu Meet Proc Chem Spec Manuf Assoc; New York. 8 pp.

Snow, R.W., Bradley, A.K., Hayes, R., Byass, P. & Greenwood, B.M. 1987. Does woodsmoke protect against malaria? Ann. Trop. Med. Parasitol. 81: 449-451.

Snow, R.W., Jawara, M., Curtis, C.F. 1987. Observations on Anopheles gambiae Giles s. l. (Diptera: Culicidae) during a trial of permethrin-treated bed nets in The Gambia. Bull. Entomol. Res. 77: 279-286.

Snow, R.W., Phillips, A., Lindsay, S.W. & Greenwood, B.M. 1988. How best to treat bed nets with insecticide in the field. Trans. R. Soc. Trop. 82: 647-648.

Snow, R.W., Rowan, K.M. & Greenwood, B.M. 1987. A trial of permethrin-treated bed nets in the prevention of malaria in Gambian children. Trans. R. Soc. Trop. 81: 563-567.

Snow, R.W., Rowan, K.M., Lindsay, S.W. & Greenwood, B.M.A 1988. Trial of bed nets (mosquito nets) as a malaria control strategy in a rural area of The Gambia, West Africa. Trans. R. Soc. Trop. 82: 212-215.

Soliman, S.A. & Cutkomp, L.K. 1963. A comparison of chemoreceptor and whole-fly responses to DDT and parathion. J. Econ. Entomol. 56: 492-494.

Sotiroff-Junker, J.A. 1978. Bibliography on the behavioral, social, and economic aspects of malaria and its control. WHO Offset Publ. 42: 1-94.

Status of malaria eradication in the Americas 1971. Nineteenth report. PAHO, 20th Meeting; 1971 Sep; Washington, D.C. 19: 1-139.

Su, N., Tamashiro, M., Yates, J.R. & Haverty, M.I. 1982. Effect of behavior on the evaluation of insecticides for prevention of or remedial control of the Formosan subterranean termite. J. Econ. Entomol. 75: 188-193.

Suarez, M.F., Quinones, M.L., Palacios, J.D. & Carrillo, A. 1990. First record of DDT resistance in Anopheles darlingi. J. Am. Mosq. Control Assoc. 6: 72-74.

Subbarao, S.K., Vasantha, K. & Sharma, V.P. 1988. Responses of Anopheles culicifacies sibling species A and B to DDT and HCH in India: implications in malaria control. Med. Vet. Entomol. 2: 219-223.

Sudomo, M., Baroji & Sustriayu, N. 1985. Chemical control on malaria vector Anopheles aconitus in central Java, Indonesia. Southeast Asian J. Trop. Med. Publ. Hlth. 16: 153-162.

Sundaram, R.M., Sadanand, A.V. & Narayanasamy, G. 1989. Susceptibility status of vectors of Japanese encephalitis to insecticides in South Arcot and Tirunelveli districts of Tamil Nadu. J. Commun. Dis. 21: 218-221.

Sundararaman, S. 1958. The behavior of Anopheles sundaicus Rodenwaldt in relation to the application of residual insecticides in Tjilatjap, Indonesia. Indian J. Malariol. 12: 129-156.

Sundararaman, S., Soeroto, R.M. & Siran, M. 1957. Vectors of malaria in mid-Java. Indian J. Malariol. 11: 321-338.

Suwonkerd, W., Amg-Ung, B., Rimwangtrakul, K., Wongkattiyakul, S., Kattiyamongkool, B., Chitraprop, U. & Takagi, M. 1990. A field study on the response of Anopheles dirus to DDT and fenitrothion sprayed to huts in Phetchabun province, Thailand. Trop. Med. 32: 1-5.

Tamaki, Y. 1979. Complexity, diversity, and specificity of behavior-modifying chemicals in Lepidoptera and Diptera. In: Shorey, H.H. & McKelvey, J.J., editors. Chemical Control of Insect Behavior: Theory and Application. New York, N.Y. John Wiley & Sons.

Tarzwell, C.M. & Fisk, F.W. 1947. Observations on the night-time resting and biting habits of anopheline mosquitoes in DDT-treated and -untreated buildings. Publ. Hlth. Rept. 62: 84-94.

Taylor, B. 1975. Changes in the feeding behavior of a malaria vector, Anopheles farauti Lav., following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. Trans. R. Entomol. Soc. London. 127: 277-292.

Taylor, R.N., Hill, M.N., Stewart, D.C., Slatter, R. & Gichanga, M. 1981. A field evaluation of permethrin (OMS 1821) and NRDC 161 (OMS 1998) for residual control of mosquitoes. Mosq. News 3: 423-434.

Thomas, G., Schomaker, C.H., Been, T.H., Van Der Berg, M.J. & Prijs, H.J. Host finding and feeding in Hydrotaea irritans (diptera:muscidae): the role of chemical senses. Vet. Parasitol. 18: 209-221.

Tillman, R. E. 1981. (New York Botanical Garden, Cary Arboretum). Environmental guidelines for irrigation. U.S. Man and the Biosphere Program and USAID. 74 pp.

Tomaszunas, S. 1966. Walka z zimnica demokratycznej republice Wietnamu [Malaria eradication in democratic republic of Vietnam]. Bull. Inst. Marine Med. Gdansk. 17: 51-58.

Toure, Y. T. 1989. Vector study and vector control in the control of malaria and babesiosis. Trans. R. Soc. Trop. 83(supplement): 39-41.

Trape, J. F. & Zoulani, A. 1987. Malaria and urbanization in Central Africa: the example of Brazzaville. Part 2: results of entomological surveys and epidemiological analysis. Trans. R. Soc. Trop. 81(suppl): 10-18.

Trapido, H. 1954. Recent experiments on possible resistance to DDT by Anopheles albimanus in Panama. Bull. WHO 11: 885-889.

Trapido, H. 1962. Modified response of Anopheles albimanus to DDT residual house spraying in Panama. Am. J. Trop. Med. Hyg.1: 853-861.

Traub, R. & Elisberg, B.L. 1962. Field tests on diethyltoluamide [deet], a highly effective repellent against mosquitoes in the nipah palm-mangrove swamps in Malaya. Pac. Ins. 4: 303-313.

Travis, B.V. Known factors causing variations in results of insect repellent tests. Mosq. News 10: 126-132.

Travis, B.V., Morton, F.A., Jones, H.A. & Robinson, J.H. 1949. The more effective mosquito repellents tested at the Orlando, Fla., laboratory, 1942-47. J. Econ. Entomol. 42: 686-694.

U.S. General Accounting Office 1982. Malaria control in developing countries: where does it stand? What is the U S. role? ID-82-27. (Report to the administrator, Agency for International Development).

UNDP/World bank/WHO. 1988. Report on a technical consultation on research in support of malaria control in the Amazon Basin. 27 pp. (TDR/FieldMal/SC/Amaz (88.3)).

Ungureanu, E. M. & Gheorghiu, T. 1980. Comparative studies on the behaviour of a population of Anopheles maculipennis under the influence of DDT and malathion, using the multiple-response test-apparatus. WHO/VBC80.774: 1-7.

Ungureanu, E. M. & Teodorescu, C.1963. Observations on the irritability of mosquitoes to DDT. India J. Malariol. 17: 47-53.

Urbino, C.M., Digma, F. & Abinoja, B. 1961. Behavior pattern of adults of Anopheles minimus flavirostris Ludlow. Philippine J. Sci. 90: 371-376.

Uvo, D. & Dias, J. 1961. Suscetibilidade do Anopheles darlingi e outros anofelinos ao DDT e ao dieldrin no municipio de Aracatuba. Arq. Hig. Saude. Publica. 26: 133-135.

Valentino, L. & Smiraglia, C.B. 1965. Eta fisiologica di Anopheles labranchiae in aree diverse di Sicilia in rapporto all'epoca del trattamento con DDT. Rivista di Malariologia. 44: 191.

Van Den Assem, J.A. 1959. Window-trap hut experiment to test the effects of dieldrin under local conditions in the Merauke area (Netherlands New Guinea). Trop. Geogr. Med. 11: 32-43.

Van Someren, E.C.C., Heisch, R.B. & Furlong, M. 1958. Observations on the behavior of some mosquitos on the Kenya Coast. Bull. Entomol. Res. 49: 643-660.

Van Thiel, P.H. & Metselaar, D.A. 1957. Pilot project of residual spraying as a means of controlling malaria transmitted by anophelines of the punctulatus group in Netherlands New Guinea. Acta Leidensia. 27: 158-179.

Van Thiel, P.H. 1951. The repellent effect of insecticides, especially of contact insecticides. Doc. Neerl. Indones. Morb. Trop. 3: 117-125.

Vargas, L. 1962. Consideraciones sobre la accion irritante del DDT para los Anopheles. Revista de la Sociedad Mexicana Historia Natural 23: 187-190.

Vargas, L. 1973. El fenomeno de la resistencia a insecticidas en Anopheles transmisores de paludismo [The phenomenon of insecticide resistance in the anopheline vectors of malaria]. Salud Publica de Mexico 15: 21-27.

Vargas, L. 1976. Criterio epidemiologico para valorar las pruebas de susceptibilidad de Anopheles a insecticidas y la importancia del fenomeno de la excitation que produce el DDT [Epidemiological criteria for assessing the susceptibility of Anopheles to insecticides and the significance of the irritant effect of DDT]. Bol Dir Malariol Saneam Ambient. 16: 363-366.

Veloso, H.P., Moura, J.V. & Klein, R.M. 1956. Delimitacao ecologica dos anofelineos do subgenero Kerteszia na regiao costeira do sul do Brasil. Memorias do Instituto Oswaldo Cruz. 54: 517-548.

Venhuis, W.G. 1941. De vindplaatsen van geinfecteerde Anopheles maculatus tijdens een epidemie in Oost Java. Geneeskundig Tijdschrift Voor Nederlansch-Indie 81: 2178-2189.

Vladimirova, V.V. 1965. Repellents against various kinds of blood-sucking dipterous insects (literature review)]. Med. Parazitol. (Mosk) 34: 340-346.

Vladimirova, V.V. 1970. Sensitivity to repellents of some species of mosquitoes and horseflies in relation to their ecology. Report 3. Med Parazitol (Mosk) 39: 591-598.

Vladimirova, V.V. 1970. [The susceptibility of mosquitoes to repellents in relation to their physiological condition. Communication 2]. Med Parazitol (Mosk) 39: 49-53.

Walch, E.W. 1932. Het verband tusschen de voorkeur van Nederlandsch-Indische anophelinen voor menschen-of dierenbloed en haar gevaarlijkheid (Tweede mededeeling). Geneeskundig Tijdschrift voor Nederlandsch-Indie 72: 682-709.

Wharton, R.H. 1951. The habits of adult mosquitoes in Malaya: 1. Observations on anophelines in window-trap huts and at cattle-sheds. Ann. Trop. Med. Parasitol. 45: 141-154.

White, G.B. 1969. Blood feeding habits of malaria vector mosquitoes in the South Pare district of Tanzania 10 years after cessation of a dieldrin residual spraying campaign. WHO/VBC69.144: 1-15.

White, G.B. 1973. The insect-repellent value of Ocimum SPP. (Labiatae): Traditional anti-mosquito plants. East Afr. Med. J. 50: 248-252.

Wijers, D.J.B. & Kiilu, G. 1984. Studies on the vector of kala-azar in Kenya, part 8. The outbreak in Machakos district; epidemiological features and a possible way of control. Ann. Trop. Med. Parasitol. 78: 597-604.

Wilkinson, R.N., Miller, T.A. & Esah, S. 1970. Anthropophilic mosquitoes in central Thailand, with notes on Anopheles balabacensis Baisas and malaria. Mosq. News 30: 146-48.

Wilson, H.G., Weidhaas, D.E. & LaBrecque, G.C. 1973. Toxicity of insecticide residues to Anopheles quadrimaculatus and effects on resting behavior. Mosq. News 33: 539-542.

Winteringham, F.P.W. 1963. Action and inaction of insecticides. PANS 9: 97-117.

Wood, R.J. 1961. Selective effects of insecticides on mosquitoes. Genetica Agraria. 14: 267-287.

World Health Organization 1970. Instructions for determining the susceptibility or resistance of adult mosquitos to organochlorine insecticides. WHO Tech. Rep. Ser. 443.

World Health Organization 1981. Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides. WHO/VBC81.6: 1-4.

World Health Organization 1981. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate, and carbamate insecticides- diagnostic test. WHO/VBC81.806: 1-7.

World Health Organization 1981. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate, and carbamate insecticides- establishment of the base-line. WHO/VBC81.805: 1-7.

World Health Organization 1981. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC81.807: 1-6.

World Health Organization 1981. Meeting of directors of WHO collaborating centres on the evaluation and testing of new pesticides. WHO/VBC88.957.

World Health Organization 1992. Vector resistance to pesticide. Fifteenth report of the WHO Expert Committee on vector biology and control. WHO Tech. Rep. Ser. 818.

Wright, J.W., Fritz, R.F. & Haworth, J. 1972. Changing concepts of vector control in malaria eradication. Annu. Rev. Entomol. 17: 75-102.

Wright, R. H. 1962. The attraction and repulsion of mosquitoes. World. Rev. Pest Cont. 1: 1-12.

Wright, R.H. & Kellogg, F.E. 1964. Host size as a factor in the attraction of malaria mosquitoes. Nature 202: 321-322.

Wyler, D.J. 1983. Malaria--resurgence, resistance, and research. New. Engl. J. Med. 308: 875-878.

Yang, T.H. 1983. A review of literature on Anopheles balabacensis balabacensis. WHO/VBC83.873: 1-30.

Yasutomi, K., Urabe, K. & Wada, Y. 1986. Insecticide-resistance of Anopheles sinensis and Culex tritaeniorhynchus in Saitama Prefecture, Japan. Jap. J. Sanit. Zool. 37: 357-362.

Young, M.D. & Johnson, T.H. 1949. A malaria study of Liberia. J. Nat. Mal. Soc. 8: 247-266.

Yust, H.R. 1947. DDT to control Anopheles farauti on Espiritu Santo, New Hebrides Islands. J. Econ. Entomol. 40: 762-768.

Yust, H.R. 1947. DDT to control Anopheles farauti on Espiritu Santo, New Hebrides Islands. J. Econ. Entomol. 40: 762-768.

Zafar-Latif, A., Strickland, G.T., Fox, E., Khaliq, A.A. & Chowdry, M.A. 1985. Seasonal fluctuation of malaria in four selected villages of the Punjab. Pakistan. J. Med. Res. 24: 53-61.

Zahar, A.R. & Davidson, G. 1973. The practical implication of resistance of malaria vectors to insecticides. WHO/VBC73.448: 1-12.

Zahar, A.R. 1974. Review of the ecology of malaria vectors in the WHO eastern Mediterranean region. Bull. WHO 50: 427-440.

Zahar, A.R. 1984. Vector control operations in the African context. Bull. WHO 62(suppl): 89-100.

Zhogolev, D. T. 1968. [On the sensitivity to repellents of Anopheles pulcherrimus Theo. and some other species of mosquitoes]. Med Parazitol (Mosk) 37: 493-495.